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50 anni di ricerca scientifica e di formazione nel campo della tecnologia e della legislazione farmaceutiche

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1973-2023 – 50th ADRITELF Anniversary

IV Convegno della divisione di Tecnologia Farmaceutica - SCI

Trieste September 11-13, 2023

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Monday, September 11

14.00-14.30	**Opening Ceremony**	
14.30-14.45	**Prof. Joerg Breitkreutz**, Heinrich Heine University Duesseldorf and APV, Mainz, Germany	
	Plenary Lecture	
Chairperson	*Anna Fadda*	
14.45-15.25	Prof. Elias Fattal, University Paris Sud – Saclay *Breaking down barriers in designing nanomedicines for the treatment of inflammatory diseases*	
	Keynotes	
	Room A – Sala Tergeste	**Room B – Sala Zodiaco**
	Session 1	**Session 2**
Chairpersons	*Donatella Paolino – Elisabetta Gavini*	*Silvia Rossi – Francesca Ungaro*
15.30-15.55	Innovation in pharmaceutical nanotechnologies: tailoring the design of nanomedicines *Giovanni Tosi, University of Modena and Reggio Emilia*	Surfactant-based "soft" nanocarriers: drug delivery and more *Maria Jole Carafa, University Sapienza*
15.55-16.25	***Coffee Break***	
16.25-16.50	Nose-to-brain drug delivery systems and formulative approaches of photosensitizers *Giovanna Rassu, University of Sassari*	Dual function surfactants for pharmaceutical formulations: the case of alkyl biguanides *Giulia Bonacucina, University of Camerino*
16.50-17.15	Brain delivery of therapeutics for neurodegenerative diseases *Antonio Di Stefano, University of Chieti G. d'Annunzio*	Host-directed strategies and sustainable - green technologies to fight infectious and inflammatory diseases *Maurizio Ricci, University of Perugia*
17.15-17.40	Prodrugs, micro or nanoparticulate systems and nasal administration as non-invasive approaches against brain diseases and airborne infections. Loco-regional therapy of cancer *Alessandro Dalpiaz*, University of Ferrara	New horizons for drug delivery to the airways *Fabio Sonvico, University of Parma*
17.40-18.05	Tailor made biocompatible polymers for nanomedicines, theranostics and regenerative medicine *Gennara Cavallaro, University of Palermo*	The role of Italian community pharmacy in primary secondary and tertiary prevention: ten years of experience *Paola Brusa, University of Turin*
18.05-19.00	**Poster session**	
19.00-19.45	**Welcome cocktail**	

Tuesday, September 12

Plenary Lectures

Chairperson	*Paolo Caliceti*
9.00-9.40	Prof. Dan Peer, University of Tel Aviv *Play God with RNA*
Chairperson	*Ruggero Bettini*
9.40-10.20	Prof. Stefaan De Smedt, University of Ghent *Nucleic Acid Delivery: on recent findings from the Ghent Research group on Nanomedicines*
10.20-10.50	***Coffee break***

Keynotes

	Room A – Sala Tergeste **Session 3**	**Room B – Sala Zodiaco** **Session 4**
Chairpersons	*Paolo Blasi - Ida Genta*	*Aurelie Schoubben – Cristina Bonferoni*
10.50-11.15	Multicargo polymeric nanoparticles to overcome resistance in cancer chemotherapy *Fabiana Quaglia, University of Naples Federico II*	From scleroglucan to 3D-bioprinting: a 25-year story *Maria Antonietta Casadei, University Sapienza*
11.15-11.40	Core and surface engineering of "smart" drug nanocarriers *Stefano Salmaso, University of Padova*	Design and development of drug delivery systems based on swellable hydrophilic polymers *Alessandra Maroni, University of Milan*
11.40-12.05	Less is more. From liposomes to nanocrystals *Chiara Sinico, University of Cagliari*	Improving on nature: successfully nanocarriers for the optimisation of biopharmaceutical properties of natural compounds and plant extracts *Maria Camilla Bergonzi, University of Florence*
12.05-12.30	Zein as a versatile biomaterial: new perspectives for innovative applications *Donato Cosco, University Magna Graecia of Catanzaro*	Progress of pharmaceutical technological and cosmetic research at Genoa university *Gabriele Caviglioli, University of Genoa*
12.30-14.30	***Lunch Break***	

Tuesday, September 12

Plenary Lecture

Chairperson *Patrizia Santi*

14.30-15.10 Prof. Sophia Antimisiaris University of Patras
Targeted liposomes for drug delivery applications, systemic and local administration

Keynotes

	Room A – Sala Tergeste	**Room B – Sala Zodiaco**
	Session 5	**Session 6**
Chairpersons	*Stefano Giovagnoli – Elisabetta Esposito*	*Piera di Martino – Gaia Colombo*
15.10-15.35	Technological platforms for ocular drug delivery *Rosario Pignatello, University of Catania*	Pharmaceutical technology 2.0: innovative perspectives for the formulation of drugs at the molecular-level *Dritan Hasa, University of Trieste*
15.35-16.00	Enhancing drug transport across biological barriers: polymeric micelles for skin, buccal and ocular drug delivery *Sara Nicoli, University of Parma*	New insights into solid lipid microparticles for oral administration produced by spray congealing technology *Nadia Passerini, University of Bologna*
16.00-16.25	Past innovations and future perspectives in ophthalmic drug delivery-an update on nanosystems *Patrizia Chetoni, University of Pisa*	From pharmaceutical technique to pharmaceutical technology in 50 years at the university of Padua *Nicola Realdon, University of Padova*
16.25-16.55	***Coffee Break***	
	Room A – Sala Tergeste	**Room B – Sala Zodiaco**
	Session 7	**Session 8**
Chairpersons	*Paolo Giunchedi – Silvia Arpicco*	*Carmelo Puglia – Anna Rita Bilia*
16.55-17.20	Electrospun nanofiber matrices: a platform for soft tissue repair, regeneration and drug delivery *Bice Conti, University of Pavia*	When the pharmaceutical technology meets regulatory science *Paola Minghetti, University of Milan*
17.20-17.45	Can lipid-based nanosystems be a tool to overcome skin barrier? *Rita Cortesi, University of Ferrara*	Pharmaceutical technology platforms for the development of tailored paediatric dosage forms *Nunzio Denora, University of Bari "Aldo Moro"*
17.45-18.10	An evolution of mucoadhesive drug carriers based on chitosan and cyclodextrin derivatives *Anna Maria Piras, University of Pisa*	The versatile role of cyclodextrins in drug delivery *Francesca Maestrelli, University of Florence*
18.10-18.25	***Journal of Controlled Release***	
18.25-19.00	***Poster session***	
20.00	**Social Dinner**	

Wednesday, September 13

Plemary Lectures

Chairperson *Paolo Caliceti*

9.00-9.40 Prof. Andreas Bernkop-Schnürch, University of Innsbruck
"Lipid-based Nanocarriers for Oral peptide Drug Delivery: Hype or Hope?"

Chairperson *Ruggero Bettini*

9.40-10.20 Prof. Maria José Alonso, University of Santiago de Compostela
"Advanced therapies and Personalized Medicine: The role of Pharmaceutical Nanotechnology"

10.20-10.50 ***Coffee break***

Keynotes

	Room A – Sala Tergeste	**Room B – Sala Zodiaco**
	Session 9	**Session 10**
Chairpersons	*Donato Cosco – Carlotta Marianecci*	*Pietro Matricardi – Lucia Zema*
10.50-11.15	The landscape of pharmaceutical technology in Turin University: focus on nanotechnology strategies *Roberta Cavalli, University of Turin*	Lipid nanoparticles for RNA delivery: potentialities and strategies to foster their clinical use *Giuseppe De Rosa, University of Naples Federico II*
11.15-11.40	Antibody-drug conjugates and immunoliposomes against her2 positive cancers. *Gianfranco Pasut, University of Padua*	Liposomal therapeutics: the never end story in drug delivery *Christian Celia, University of Chieti G. d'Annunzio*
11.40-12.05	Macro- micro- and nano drug delivery systems @ DIFARMA - University of Salerno *Pasquale Del Gaudio, University of Salerno*	Multifunctional and bioactive polymers for tissue engineering and drug delivery *Giuseppina Sandri, University of Pavia*
12.05-12.30	Molecularly Imprinted Synthetic Material Antibodies (MISMAs) as artificial counterpart of conventional antibodies in molecular recognition *Francesco Puoci, University of Calabria*	3D printing in pharmaceutics: how to use it? *Luca Casettari, University of Urbino Carlo Bo*

12:30 ***Lunch***

Monday, September 11

14.00-14.30 **Opening Ceremony**

14.30-14.45 **Prof. Joerg Breitkreutz**,
Heinrich Heine University Duesseldorf and APV, Mainz, Germany

Chairperson *Anna Fadda*

14.45-15.25 Prof. Elias Fattal, University Paris Sud – Saclay
Breaking down barriers in designing nanomedicines for the treatment of inflammatory diseases

PLENARY LECTURE

Breaking down barriers in designing nanomedicines for the treatment of inflammatory diseases

Elias Fattal
Institut Galien Paris-Saclay, UMR CNRS 8612, Orsay, France

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Nanomedicines are today strongly considered for drug delivery in inflammatory diseases since they can cross leaky endothelium reaching the inflammatory sites and release their payload therein. Pegylated liposomes were also shown in rheumatoid arthritis (RA) to be taken up by myeloid cells and transported to inflamed joints. We have designed novel pegylated dexamethasone nanoparticles (NPs). They were obtained using dexamethasone palmitate and DSPE-PEG2000 and characterized for their physicochemical and biopharmaceutical properties. We have evaluated their therapeutic efficacy and joint targeting effect in collagen-induced arthritis (CIA) mice model. Three NPs IV injections at 1mg/kg (eq.DXM) significantly improved CIA symptoms compared to saline or free drug. Fluorescent NPs proved their specific accumulation into inflamed joints. More recently, we have attempted to correct the increased expression of miR-155 in rheumatoid arthritis, which could be responsible for impaired monocyte polarization to anti-inflammatory M2-like macrophages. In this study, two pre-clinical models of RA, the CIA and the K/BxN Serum-Transfer-Arthritis (STA), were used to examine the therapeutic potential of antagomiR-155-5p, entrapped within pegylated liposomes with protamine: nucleic acid core, in the resolution of arthritis and re-polarization of monocytes towards anti-inflammatory M2 phenotype. We demonstrated the biodistribution of fluorescently tagged-pegylated liposomes to inflamed joints 1 hour after injection in arthritic mice. IV injection of the liposomes containing antagomiR-155-5p decreased arthritis score and paw swelling. Moreover, the treatment restored bone marrow monocyte defects in anti-inflammatory macrophage differentiation without any significant functional change in other immune cells, including splenic B and T cells.

Sepsis and acute lung injury can be described as an immune disorder caused by an infection characterized by a cytokine storm. We synthesized cationic phosphorus dendrons and dendrimers platform to deliver TNF-α siRNA in mice models of LPS-induced lung injury. The most efficient dendrimers to complex siRNA are from generation 3 and possess pyrrolidinium as terminal protonated amino end-groups. Dendriplexes were able to promote cellular uptake. Moreover, they led to a good inhibition of TNF-α in the lipopolysaccharide (LPS)-activated mouse macrophage cell line RAW264.7. The highest TNF-α silencing effect (80%) was confirmed in vivo in a murine model of acute lung inflammation induced by LPS after nasal delivery of dendriplexes (v/s free siRNA). The same siRNA was also entrapped in lipid NPs, and we monitored in vitro the intracellular trafficking and their anti-inflammatory effect over time using several imaging techniques and cellular assays. Results suggest that while lipid NPs internalization happens almost instantaneously, SiRNA release and inhibitory effect started at around 16h, which is compatible with emergency treatment. In vivo in the LPS-induced lung injury in mice, lipid nanoparticles carrying siRNA were taken up widely by neutrophils at first and later by macrophages, two main actors of the cytokine storm, which in the case of treatment with anti-TNF-α silencing by siRNA was reduced significantly. These studies demonstrate the high potential of nanomedicines in the delivery of anti-inflammatory drugs and nucleic acid-targeting signaling pathways involved in inflammation.

Elias Fattal is a full professor in Drug Delivery Science at the University of Paris-Saclay and has been president of APGI from 2003 to 2010. He received his Pharmacy Degree (1983) and Ph.D. (1990) from the University of Paris-Sud and followed an internship in Hospital Pharmacy at the University of Lille (1984-1986). After a post-doctoral position at the University of California, San Francisco, in 1990-1991, he became an Associate Professor (1992) and full Professor at the University of Paris-Saclay (2000). He has been the head of the Institut Galien Paris-Saclay (2010-2019). He has made fundamental and applied contributions to the fields of drug delivery using nanotechnologies for targeted or local delivery of drugs and nucleic acids. He has recently focused on lung nanotoxicity and the design of nanoparticle-based delivery systems to deliver anti-inflammatory drugs and nucleic acids. One of his patents led to Calixarene® Cevidra, a cream for treating external actinide contamination. Prof. Fattal has received the Pharmaceutical Sciences World Congress (PSWC) Research Achievement (2007), the Controlled Release Society fellow award (2016), was awarded in 2016 by the French Academy of Sciences for his research at the interface of chemistry and biology and more recently in 2018 received the Maurice-Marie Janot Award. He serves on the editorial board of several scientific journals and is a member of the French Academy of Pharmacy, the French Academy of Medicine, and the European Academy of Sciences.

Monday, September 11

Session 1

Room A - Sala Tergeste

Chairpersons *Donatella Paolino – Francesco Cilurzo*

15.30-15.55 Innovation in pharmaceutical nanotechnologies: tailoring the design of nanomedicines

Giovanni Tosi, University of Modena and Reggio Emilia

15.55-16.25 Coffee break

16.25-16.50 Nose-to-brain drug delivery systems and formulative approaches of photosensitizers

Giovanna Rassu, University of Sassari

16.50-17.15 Brain delivery of therapeutics for neurodegenerative diseases

Antonio Di Stefano, University of Chieti G. d'Annunzio

17.15-17.40 Prodrugs, micro or nanoparticulate systems and nasal administration as non-invasive approaches against brain diseases and airborne infections. Loco-regional therapy of cancer

Alessandro Dalpiaz, University of Ferrara

17.40-18.05 Tailor made biocompatible polymers for nanomedicines, theranostics and regenerative medicine

Gennara Cavallaro, University of Palermo

18.05-19.00 Poster session

19.00-19.45 Welcome cocktail

INNOVATION IN PHARMACEUTICAL NANOTECHNOLOGIES: TAILORING THE DESIGN OF NANOMEDICINES

G. Tosi, B. Ruozi, J.T. Duskey, I. Ottonelli, F. Forni, M.A. Vandelli,

TeFarTI Laboratories, Department of Life Sciences, University of Modena and Reggio Emilia

The development of advanced pharmaceutical carriers for the delivery and targeting of different kinds of active molecules has changed drastically over the last 20 years. This is because scientists have begun ameliorating their design and overcoming possible limitations by planning more flexible drug delivery systems (DDSs), and in particular Nanomedicines (NMeds). Over the years, the growth of technological platforms dedicated to drug delivery has been faced by a number of research groups. Within our Te.Far.T.I. labs, advanced research was performed on tailoring the design of DDSs by producing and characterizing different types of DDSs, dedicating major attention to the characteristics of the drugs to be delivered, key-features of selected pathologies, and our final biological and pharmaceutical goals to be reached. The advancement of our research was mainly focused on the passage from micro to nanosized systems. Initially, a strong focus was devoted to the development of microparticles made of biocompatible and biodegradable materials, such as gelatin [1], with the final aim of precisely controlling the drug release of different kinds of active molecules. Then, always keeping biodegradability and biocompatibility as pivotal features, the research advanced towards nanosized DDSs, lipidic-based nanomedicine, such as liposomes [2], or polymeric-based DDSs, such as nanoparticles [3]. The development of DDS design started by critically analyzing and investigating the major advantages and criticisms in the production, preparation, and characterization NMeds created from different materials [3-6]. Following the progress of advanced technologies (for example atomic force- and confocal- microscopy, photon correlation spectroscopy, etc…), a large step towards a deeper knowledge of the overall characteristics and their effects on pharmaceutical properties was achieved.

Also critical, the planning of NMeds is strongly dependent on the type and properties of the active molecules to be delivered, the barriers to be crossed, and the overall pharmaceutical outcome desired. Thus, over the years, a broad platform of NMeds have been optimized for the delivery of a wide range of biotherapeutics ranging from small molecules to enzymes and gene material [8-16]. Furthermore, to achieve active targeting, surface engineering of NMeds is needed. This has been improved by adapting/creating protocols to surface decorate NMeds with different kinds of ligands, such as fluorescent probes, small peptides, and antibodies [17-20].

Designing a NMed is not only based on drug properties or their delivery, but must also take into consideration the transition from a lab environment to clinical development. This is particularly important in terms of industrial production [21]; thus, research must heavily consider future scale-up issues, flexibility of the platform for the large quantity production of high quality products following FDA guidelines.

Finally, the design of an NMed and its function should/must be adaptable to *in vitro/in vivo* readouts; thus, detailed studies and understanding on NMed stability, safety, and their intracellular/intercellular destiny was conducted, thus ameliorating the overall properties of NMeds [23]. Regarding the application of these NMeds towards unmet clinical needs, our research was mainly focused and dedicated to Cancers (hematological and solid ones), Rare and Genetic Diseases, and Brain Diseases (from neurodegenerative to brain tumors), with a number of molecules delivered via the use of selective and targeted NMeds The sum of our work has helped create a solid basis of knowledge in terms of the pharmaceutical possibilities of NMeds, and has offered promising results in preclinical experiments [14-17,20,23].

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NOSE-TO-BRAIN DRUG DELIVERY SYSTEMS AND FORMULATIVE APPROCHES OF PHOTOSENSITIZERS

G. Rassu, C. Serri, E. Gavini, P. Giunchedi

Università degli Studi di Sassari, Dipartimento di Medicina, Chirurgia e Farmacia

In 2005, the Research Group of Pharmaceutical Technology of the University of Sassari published the first article on microparticulate delivery systems designed for the nasal administration of an antiemetic drug [1]; it was the beginning of an interesting and fruitful research line. Since then, different nasal drug delivery systems were designedand in vitro, ex vivo and in vivo characterised in collaboration with the Italian (University of Ancona, Ferrara, Pavia, Pisa) and International Universities (University of Oslo, Luleå University of Technology, Thomas Jefferson University, Ege University, CESPU in Gandra). The nasal route was explored as a potential alternative route to oral or parenteral administration for systemically active drugs [1-3] as well as for obtaining a direct nose-to-brain transport of drugs [4-16]. The design of nasal dosage forms has to consider the anatomic and physiologic characteristics of nasal mucosa and more particularly the rapid mucociliary clearance that limits the time available for drug adsorption. For that, spray-dried microspheres based on chitosan, its salts (chitosan chloride) and its derivatives (chitosan glutamate, methyl chitosan, methylpyrrolidinone chitosan) alone or in combination with alginate, was select as delivery systems of different drugs (metoclopramide, carbamazepine, N6-cyclopentyladenosine, rokitamycin, dopamine, zolmitriptan, deferoxamine). Chitosan is one of the most used excipients for nasal formulations due to its mucoadhesive and penetration enhancement properties [17]. Microspheres based on chitosan salts absorbed quickly a very small amount of aqueous fluid which is enough to cause swelling, gelling, and then dissolving, whereas microparticles based on chitosan base needed more volume of water and remained on the surface as swelled and insoluble particles [4,7]. When the microspheres absorbed water from the mucus and swelled, the epithelial cells were dehydrated and caused the opening of the tight junctions; thus, it increased the paracellular absorption of drugs. The enhancing effect was rapid and reversible. In particular, microspheres based on chitosan chloride appeared able to promote the *in vivo* nose to brain uptake of deferoxamine and N6-cyclopentyladenosine (CPA),minimizing systemic drug exposure [11,14]. Compared with methyl-β-cyclodextrin, chitosan microparticlesallowed to obtain a selective distribution of CPA in cerebrospinal fluid after nasal administration respect to the bloodstream (Figure 1) [14].

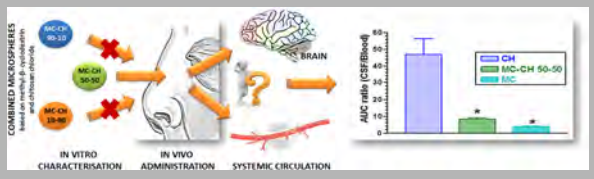

Figure 1. The role of chitosan and methyl-β-cyclodextrin in nasal microspheres on in vivo CPA bioavailability [14].

In nasal formulations, cyclodextrins were used not just how pharmaceutical excipients (solubilizers and absorption promoters), but also as active ingredients [6,12,16]. Hydroxypropyl-β-cyclodextrin (HPCD) can directly bind β-amyloid peptides (Aβ), inhibiting amyloid fibril formation and thus decreasing the Aβ neurotoxic effect in vitro [6]. When encapsulated in chitosan-based microspheres and in vivo nasal administered for seven consecutive days toAlzheimer's disease rat model, HPCD reduced the oxidative stress and apoptotic parameters [12]. The results obtained demonstrated that HP-CD can reach the brain after nasal administration, particularly the hippocampus, where it provided a protective action against Aβ-induced excitotoxicity [12]. The suitability of new cationic β-cyclodextrin monomers andpolymers as materials for nasal formulations was also evaluated [16]. The results of cytotoxicity and cell uptake demonstrated that these cationic beta- cyclodextrins showed potential as excipients able toimprove the nasal absorption of drugs. Furthermore, amino-beta-cyclodextrin and beta-cyclodextrin soluble polymers were able to reduce oxidative damage in neuronal and epithelial cells and, thus, could be studiedas bioactive carriers or potential drugs [16].

Chitosan was also used for preparing nanoparticles [13,15]. Chitosan-coated solid lipid nanoparticles were proposed as a nasal delivery system capable of exploiting both olfactory and trigeminal nerve pathways of siRNA [13]. Chitosan nanoparticles were investigated for intranasal delivery of genistein. The nanoparticles were prepared by the ionic gelation technique by using sodium hexametaphosphate as avalid alternative cross-linkers. Nanoparticles were able to improve genistein penetration through the nasal mucosa as compared to pure genistein and to preserve PC12 cell vitality [15].

In 2013, the Research Group was involved in developing systems for the delivery of photosensitizers (indocyanine green (ICG)) used in the therapy and/or

diagnosis of tumours. In particular, polymeric platforms loaded with indocyanine green (ICG) were developed in collaboration with Department of Surgery, IRCCS Policlinico San Matteo Foundation (Pavia, Italy) with the aim to design and prepare injectable formulations for the image and loco-regionaltreatment of hepatocellular carcinoma (HCC). With this aim, several platforms were designed, were prepared and characterised such as thermosensitive chitosan/glycerophosphate (C/GP) solutions [18], in situ forming biodegradable microspheres [19,20] and poly (ethyl 2-cyanoacrylate) nanoparticles [21,22].

The leader C/GP solution had optimal rheological properties, gelling time, and capability of retaining ICG, and therefore suitable to be injected through a catheter into tumour nodules; as the solution gelled in these areas, a prolonged visualization of these nodules could be achieved in 'real time imaging' during the embolization and the following hepatic resection ofHCC [18]. Based on chitosan/ICG interaction,biocompatible indocyanine green (ICG)-loaded microspheres (CAB-CS-ICG) were prepared using amulti-step method and proposed as novel fluorescent biocompatible embolic /imaging agent in transarterial embolization (TAE). Chitosan-ICG particles were prepared via spray-dryer and then loaded into cellulose acetate butyrate (CAB) microspheres, fabricated by emulsion solvent extraction method. CAB-CS-ICG microspheres showed good injectability; they were capable to retain the dye and maintain the fluorescence selectivity for 4 weeks. In the frame of the developing polymeric systems containing the photosensitizer useful to embolize and at the same time ensure prolonged visualization, in situ forming biodegradable microspheres were also prepared using poly(ε- caprolactone) by coacervate type emulsions; the emulsions were constituted by an organic phase (N-methyl-2-pyrrolidone and Tween 80) and an oil phase (plurol diisoestearique and corn oil). After injection in the blood vessel, the microglobules of emulsions transformed in solid microspheres in situ by extraction of the organic and oily vehicles in the water [19].

Poly (ethyl 2-cyanoacrylate) nanoparticles (PECA-NPs) were prepared using an emulsion polymerization method and their potential for cancer treatment was investigated [21,22]. PECA-NPs were internalized within the cells (Figure 2) [22], and exhibited a cytotoxic effect on both 3D tumour models, hepatocellular carcinoma and kidney adenocarcinoma [21]

Finally, very recently, another photosensitizer was considered.

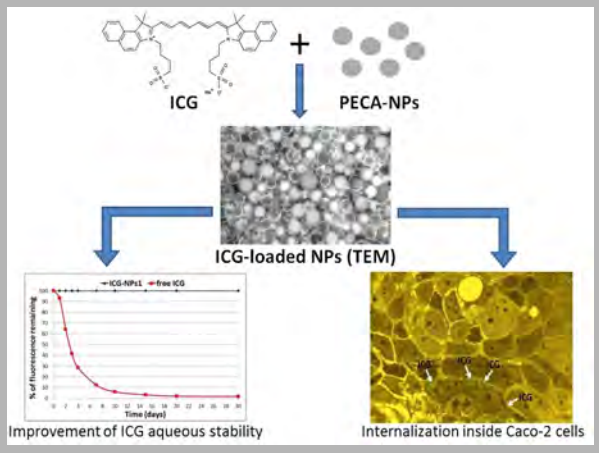

Figure 2. Interaction studies between indocyanine green loaded polymeric nanoparticles with Caco-2 cell line [22].

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BRAIN DELIVERY OF THERAPEUTICS FOR NEURODEGENERATIVE DISEASES

A. Di Stefano[a], L. Marinelli[a], M. P. Dimmito[a], E.C. Toto[a], A. Rapino[a,b], F. Palmerio[a,b], E. Spaccapaniccia [a,b], M. Lardo[a,c], A. Di Rienzo[a], I. Cacciatore[a]

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Nanotechnological approaches are often used to improve the pharmacokinetic profile of drugs that have poor gastro-intestinal absorption, low drug solubility, and rapid metabolism but also to ensure an efficient CNS delivery of many compounds. Colloidal systems have roused special interests due to many features that make them particularly intriguing as alternative carriers in the field of drug delivery systems (DDS). In combination with the narrow sizes, sterical properties obtained in stealth nanoformulations result advantageous to increase the blood circulation time of the particulate, thus reducing RES uptake and extending the contact time between the BBB and the drug, which can therefore be caught in the brain when the target is represented by CNS [1]. Such properties can improve bioavailability of drugs through several mechanisms: (1) augmenting the drug solubility and permeability, (2) overcoming the first-pass effect and the P-gp efflux, and (3) enhancing the stability in theGI tract. Several drug-loaded colloidal formulations in brain targeting and delivery showed a very lowcytotoxicity or resulted to be not-cytotoxic [2]. During the last two decades our research has been focused on the development of innovative therapeutic strategies to reduce the disease progression and improve the quality of life in patients suffering from neurodegenerative diseases such as Parkinson's (PD) and Alzheimer's (AD).

In this context, lipidic- and polymer- based DDSs have been investigated providing tools able to interact with biological systems at molecular level with a high degree of specificity, to provide neuroprotection and to facilitate the delivery of drugs and small molecules across the BBB [3,4]. Furthermore, a *green* and *eco-friendly* nanotechnology platform for brain delivery was also proposed [5].

The aim of our studies was to investigate the correlation between the *in vitro* release and the resulting pharmacokinetics of formulations optimized in terms of drug loading, size, functionalization, morphology, thermal properties, and drug release. In animal experiments, after subcutaneous (s.c.) or intraperitoneal (i.p.) injection, the resulting levels of the therapeutic moiety in rat plasma and brain tissues were measured investigating the *in vitro-in vivo* correlation (IVIVC) [6].

Novel nanoparticulate DDSs, loaded with anti-AD drugs, were successfully prepared, characterized, and tested for their brain targeting suitability. They resulted safe from a toxicological point of view. Selected new formulations reveal their capability to maintain a high Neuroglobine (Ngb) level allowing to perform a neuroprotective and antiapoptotic role, representing a valid tool in the therapeutic strategy of AD progression [7].

Studies carried out on anti-PD drugs, showed that the formulation released the bioactive compounds over nearly the same period when s.c. administered to rats as demonstrated by the Levodopa (LD) and dopamine (DA) plasma levels and DA concentration in striatal tissue. The IVIVC studies showed a good linear regression relationship between the percentage in vitro release in acetate buffer at 37 °C and the percent of AUC. Very interestingly, s.c. administration of most promising formulations resulted in sustained LD concentrations in the blood above baseline and high levels of DA neurotransmitter at the site of action, the striatum, for 4 days after its administration. A proposed depot formulation of LD releasing drug showed several advantages: (i) it reduces the required prodrug dosing frequency; (ii) it is useful for optimizing the LD pharmacokinetic profile, avoiding dangerous peaks in LD blood levels and for increasing the efficacy of LD-based PD treatment potentially limiting the associated motor syndrome side effect.

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PRODRUGS, MICRO OR NANOPARTICULATE SYSTEMS AND NASAL ADMNISTRATION AS NON INVASIVE APPROACHES AGAINST BRAIN DISEASES AND AIRBORNE INFECTIONS. LOCO-REGIONAL THERAPY OF CANCER

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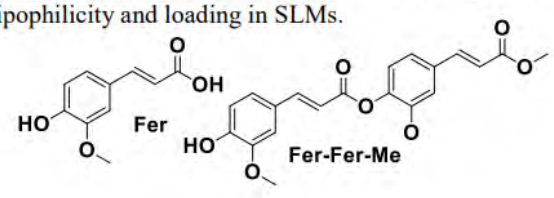
The prodrug of potential neuroactive agents can offer several advantages in order to obtain their brain targeting. Prodrugs obtained as conjugate with essential nutrients can allow the brain targeting of neuroactive agents, unable to permeate in the brain from the bloodstream, via influx transporters expressed in the blood brain barrier (BBB). For example, nipecotic acid can induce antiepileptic effects as a prodrug obtained by ester conjugation with vitamin C, being uptaken in the brain by the SVCT2 transporter [1]. Similarly, dopamine can induce antiparkinsonian effects as a prodrug obtained by conjugation with glucose, being uptaken in the brain via the GLUT1 transporter [1]. Alternatively, the prodrug approach can allow to elude the active efflux transporters (AETs) whose expression on the BBB is essential for brain protection from potential damaging molecules. Zidovudine (AZT), a reverse transcriptase inhibitor, is a substrate of AETs, and it is consequently unable to permeate in the brain or in macrophages. AZT conjugation with ursodeoxycholic acid (UDCA), a bile acid permeating into the brain, allows to obtain a prodrug (UDCA-AZT) able to elude the AETs without inhibiting them [2]. This ability allows the UDCA-AZT prodrug to permeate and remain in murine macrophages (that may constitute HIV reservoirs in the brain) with an efficiency twenty times higher than that of AZT [3]. Moreover, the prodrug UDCA-AZT can self-assemble as nanoparticle cores coated with a bile acid salt (taurocholate or ursodeoxycholate) corona without any other excipients. The taurocholate-coated nanoparticles appear able to interact with serum proteins, differently from the ursodeoxycholate-coated particles. Accordingly, the taurocholate-coated nanoparticles show in vitro uptake by murine macrophages about 70 times higher than that obtained with the free prodrug, whereas no significant uptake increase can be registered for ursodeoxycholate-coated particles. Taurocholate-coated nanoparticles may be useful against intracellular infections of the MPS system, whereas ursodeoxycholate-coated particles could have "stealth" properties in the bloodstream [4].

The nasal administration can offer a direct nose-to-brain delivery of the molecules allowing bypass the BBB. Indeed, drug molecules able to pass the mucus layer can permeate across the olfactory mucosa and directly reach the cerebrospinal fluid (CSF) or the brain parenchyma. In general, the brain uptake of nasally administered drugs is allowed by appropriate formulations able to provide several advantages, such as high local concentration of the free drug for diffusion processes. Micro or nanoparticulate systems have been designed in this aim and several neuroprotective agents have been studied concerning their brain delivery nasally, including anti-ischemic, anti-inflammatory, anti-Parkinson, antiepileptic and antimigraine drugs [1,3]. The small size and large surface area of micro and nanoparticles can limit the loading of drugs, in particular when the particulate systems are hydrophobic and the encapsulated drugs are poorly hydrophobic. When properly designed, hydrophobic prodrugs can solve these difficulties, allowing appropriate encapsulation efficiencies. As an example, the prodrug UDCA-AZT allowed zidovudine encapsulation in solid lipid microparticles (SLMs), whose nasal administration induced selective SNC targeting [5]. Very recently, the volatile geraniol was efficiently encapsulated in solid lipid nanoparticles (SLNs) only as a prodrug obtained by its ester conjugation with ursodeoxycholic acid (GER-UDCA). In this case, the nasal administration of the nanoparticles induced selective SNC targeting of the prodrug obtained by the ester conjugation of two antiparkinsonian agents [6].

Ferulic acid (Fer) is proposed for the prevention and therapy of neurodegenerative diseases. Aiming to increase the uptake and residence time of Fer in the CNS, a prodrug approach is proposed. Specifically, the prodrug has been obtained as a dimer of the drug itself, in order to increase the Fer loading on solid lipid microparticles (SLMs) designed as nasal formulation. The carboxylic moiety of a molecule of Fer was therefore esterified with the phenolic group of another Fer molecule, obtaining an ester conjugate (Fer-Fer-OH) in the absence of linkers, which avoid the production of unwanted sub-products when hydrolysed in physiological environments. Finally, the dimeric conjugate of Fer was methylated on the carboxylic moiety (Fer-Fer-Me) in order to increase its lipophilicity and loading in SLMs.



The nasal administration of the stearic acid SLMs loaded with Fer-Fer-Me is sensibly efficacious in the prodrug brain targeting, by enhancing both amounts and permanence in the rat CSF, in comparison to Fer.

Anti-inflammatory drugs in the asymptomatic initial phase of Alzheimer's disease (AD) could slow down AD progression, provided they enter the brain. Nasal administration may enable the drug direct access to brain. Flurbiprofen powders for nose-to-brain drug transport in early AD-related neuroinflammation were produced by spray drying. Flurbiprofen sodium nasal powders disclosed prompt dissolution and fast ex vivo transport across rabbit nasal mucosa [7]. Microparticles as such or soft pellets obtained by their agglomeration resulted into rapid flurbiprofen absorption in rats. Compared to intravenous flurbiprofen, the microparticles were more efficient than soft pellets at enhancing direct drug transport to CNS. Direct Transport Percentage index evidenced that more than 60% of the intranasal dose reached the brain via direct nose-to-brain transport for both powders [8].

Airborne infections by viruses like SARS-CoV-2 and other pathogens can be tackled by antinfective drug targeted delivery to infected cells. Timely drug treatment at symptom onset would stop virus spreading to lung and other organs, sparing severe disease. We are studying the formulation of repurposed drugs into microparticles for deposition into the upper airways by nasal powder administration.

29 million cases of cancers are estimated by 2040. Site-specific delivery of drugs right to target with less systemic side effects, is an ongoing challenge of chemotherapy. In this context, a cisplatin (cisPt)-loaded sodium hyaluronate polymeric film was proposed for loco-regional anticancer therapy. In a malignant pleural mesothelioma rat model, the film was applied directly on the pleural surface and effectively reduced recurrences [9]. Moreover, despite the high cisplatin (cisPt) plasma concentrations over time, organ toxicity was lower in comparison with the standard treatments (i.e., intrapleural or intravenous cisPt solution) [10]. This was attributed to the formation of complex between cisplatin and hyaluronan [11]. Currently, our studies pursue the goal of delivering cisPt/NaHA complex locally, in dosage forms other than film and in different cancers, especially in tumors where CD44 receptor is over expressed.

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Monday, September 11

Session 2

Room B - Sala Zodiaco

Chairpersons *Silvia Rossi – Francesca Ungaro*

15.30-15.55 Surfactant-based "soft" nanocarriers: drug delivery and more

Maria Jole Carafa, University Sapienza

15.55-16.25 Coffee break

16.25-16.50 Dual function surfactants for pharmaceutical formulations: the case of alkyl biguanides

Giulia Bonacucina, University of Camerino

16.50-17.15 Host-directed strategies and sustainable - green technologies to fight infectious and inflammatory diseases

Maurizio Ricci, University of Perugia

17.15-17.40 New horizons for drug delivery to the airways

Fabio Sonvico, University of Parma

17.40-18.05 The role of Italian community pharmacy in primary secondary and tertiary prevention: ten years of experience

Paola Brusa, University of Turin

18.05-19.00 Poster session

19.00-19.45 Welcome cocktail

SURFACTANT-BASED "SOFT" NANOCARRIERS: DRUG DELIVERY AND MORE

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Università degli Studi di Roma "Sapienza", Dipartimento di Chimica e Tecnologie del Farmaco

Surfactant are amphiphilic molecules bearing a hydrophilic head group and a hydrophobic tail. They are widely used in pharmaceutical products with different functions.

Vesicles formed by surfactants are known as niosomes or non-ionic surfactant vesicles (NSVs). Niosomes are a similar in terms of structure and certain physical properties to liposomes [1, 2]. The self-assembly of non-ionic surfactants into vesicles was first reported in the seventies by researchers in thecosmetic industry [3] and the research interest in surfactant-based vesicular carriers is still high(keyword-niosomes: about 800 articles published in thelast 5 years, *data retrieved from Scopus, January 2023*).

It is well known, how the surfactant structure clearly affects the size, stability, entrapment efficiency, pharmacokinetics, pharmacodynamics and targeting properties of vesicular systems. A large selection of surfactants displaying favourable properties for specific drug delivery application is readily available.

Several non-ionic surfactants are known to form vesicles.

Our research started in 1998 on Polysorbate 20 (Tween®20, TW20) niosomes: the water-soluble detergent [Hydrophobic Lipophilic Balance (HLB) value = 16.7] is not able to form niosomes in the absence of cholesterol whereas it forms stable non- ionic surfactant vesicles in the presence of equimolar cholesterol concentrations [4].

The influence of the preparation technique on the properties of the obtained structures was studied.

Vesicles with bigger dimensions and higher entrapment efficiency were obtained when sonication was carried out after the film formation.

Vesicle formation in the presence of ionic surfactants was investigated in order to evaluate the effect of charged components on vesicle dimensions, entrapment efficiency and stability. Dimethyldioctadecylammonium bromide (DDOA) and cetylpyridinium chloride (CPy) were used to introduce a positive charge in the vesicle structure, while dicetylphosphate (DCP) was used for a negativecharge. The obtained results show that the amount and the molecular structure of the ionic surfactant can affect the behaviour of the vesicles and suggest that thepresence of a net charge, whether negative or positive, can increase water uptake within the double layer.

TW20 vesicles have been proposed for several application and here are reported few examples: topical application of a novel formulation of NSVs entrapping lidocaine in the form of a free base (LID) and a hydrochloride (LIDHCl) [5]; interaction with human lung fibroblasts of beclomethasone dipropionate-loaded niosomes [6], (Figure1) and pentamidine nose-to-brain delivery in mouse model of Parkinson's disease [7], (Figure 2).

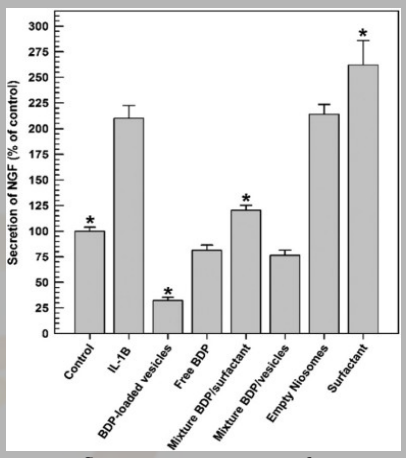

***Figure 1**. Anti-inflammatory activity of various formulations containing beclomethasone dipropionate (BDP) (at a final drug concentration of 1 μM) evaluated as inhibition of NGF secretion in primary HLF cells treated with IL-1β (as pro-inflammatory stimulating agent—10 U×ml- 1).*

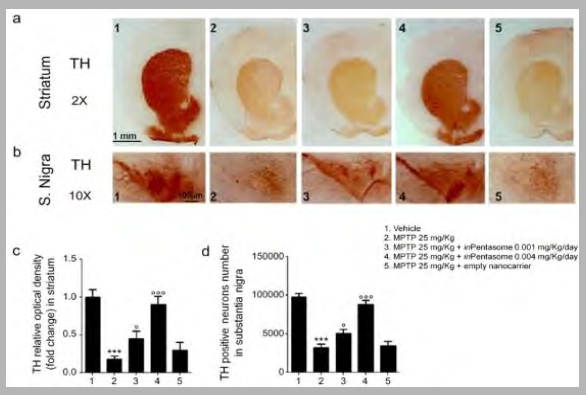

***Figure 2.** inPentasomes (Pentamidine loaded niosomes) inhibit MPTP-induced dopaminergic neuronal loss in nigrostriatal areas.*

Recently, phospholipid and surfactant vesicles have been loaded with PFC gas to obtain nanoscale echogenic bubbles (NBs).

The NBs perform as good acoustic enhancers over a wide frequency range and out of resonant conditions, as tested in both *in vitro* and *in vivo* experiments, proving to be a potential platform for the production of versatile carriers to be used in ultrasound-assisted

diagnostic, therapeutic and theranostic applications [8] (Figure 3).

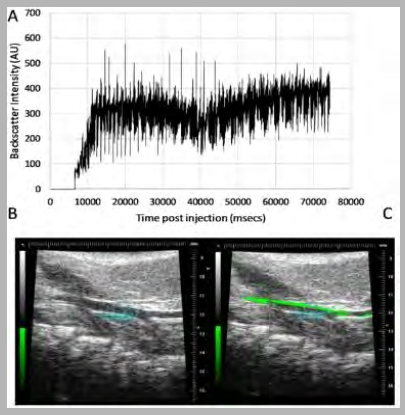

Figure 3. *A) Backscatter enhancement relative to mean reference baseline measurements, measured in carotid artery before, during and after a contrast injection of 50 µl of Span-20 nanobubbles. B) Baseline image of mouse carotid artery showing region-of-interest, outlined in blue. C) Contrast-enhanced image with green showing presence of contrast. Images acquired at 30 MHz using a Vevo 770 preclinical ultrasound scanner.*

A rational characterization of nanosystems is needed for the control of the quality of the product, which is a prerequisite for the development and clinical applications. TW20 niosomes were deeply characterized by means of different technique such as SAXS or in situ energy dispersive X-ray diffraction (EDXD) [9].

Stimuli-sensitive niosomes, which release their cargo in response to external stimuli, constitute interesting alternatives for therapies directed towards solid tumours and other spatially well-defined targets. The gradual decrease in pH experienced by niosomes that are internalised via endocytosis constitutes a very useful intrinsic stimulus and several pH-sensitive niosome formulations based on this strategy have been developed and evaluated. pH sensitivity has been conferred by addition of cholesteryl hemissucinate to TW20 niosomes, by TW20 modification or by using different surfactants [10-13].

More recently, oleic acid, a pH-sensitive monounsaturated fatty acid holding per se an antimetastatic and anti-inflammatory role in melanoma, has been added to niosomal formulation. Cytotoxicity and cellular uptake were assessed in cultured normal fibroblasts and human melanoma cell lines. Interestingly, obtained results confirm nanocarrier stability and pH-sensitivity, associated to absence of cell toxicity, efficient cellular uptake and retention. Therefore, these new pH-sensitive oleic acid-based nanostructures could represent, by combining drug delivery in pH-dependent manner with the antimetastatic potential of this fatty acid, a powerful strategy for more specific approach against metastatic melanoma [14] (Figure 4).

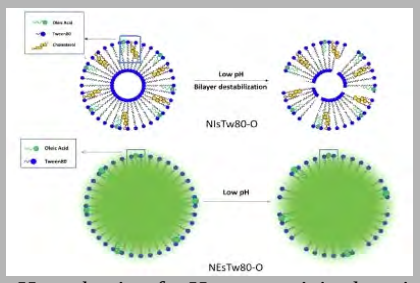

Figure 4. *Hypothesis of pH responsivity by niosomes and nanoemulsions (NEs).*

In this study a surfactant has been added also to stabilize O/W nanoemulsion obtained by oleic acid.

In O/W NEs a single oil is usually dispersed as droplets into the aqueous/continuous phase, constituting the 5–20% w/w compared to the other components. For this reason, NEs act as excellent and versatile delivery systems for poorly water-soluble drugs, which are otherwise difficult to formulate inconventional dosage forms. NEs are composed of generally recognized as safe (GRAS) grade excipients and this allows to use different surfactants and oils that can modulate or increase the drug activity [15-17].

In the last years, our expertise on surfactant-based "soft" nanocarriers prompt us to explore different fields of potential application. In an effort to replace current chemical disinfectants, natural substances with less environmental burden, such as essential oils (EO), have been envisioned as anefficient and ecologically safer alternative tool to counteract microbial growth and to eradicate biofilms. *Satureja montana* essential oil (SEO) NE formulations have been studied to evaluate their efficacy against *E. coli* strains from healthy chickens, grown in planktonic and sessile form, in order to develop an efficient product to be used in poultry farms to counteract microbial growth and biofilm formation [18]. Furthermore, EO NEs have been proposed to treat the organic fraction of urban solid waste to ameliorate biological tratments.

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DUAL FUNCTION SURFACTANTS FOR PHARMACEUTICAL FORMULATIONS: THE CASE OF ALKYL BIGUANIDES

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Biguanide is an interesting molecule whose chemical moiety can be found in the structure of several compounds with different therapeutic activities such as antidiabetic, antiviral, antimalarial, antibacterial, antimycotic, anti-HIV. Biguanide derivatives are currently employed in clinics as orally- administered hypoglycemic agents and they have been also proposed for the treatment of several other conditions (e.g. polycystic ovary syndrome, cancer, tubercolosis) [1]. Regarding the antimicrobial activity, several biguanide derivatives are known to be active against a wide range of gram positive and gram negative bacteria as well as fungi, yeasts, viruses. Indeed, a cationic bisbiguanide as chlorhexidine (and its salt form chlorhexidine digluconate) is one of the most used topical disinfectant and antiseptic agent for skin and mucosa [2]. Despite the large interest toward biguanides derivatives, no detailed studies have been conducted for the development of novel surface-active molecules bearing this chemical moiety. Indeed, only one study can be found in the literature, in which a biguanide was linked to alkyl chains of a different length to produce amphiphilic molecules [3]. According to these premises, the biguanide moiety can be exploited to design new surfactants with a dual function as surface-active molecules and antimicrobial/disinfectant agents. As such, a new series of amphiphilic compounds derived from the alkylation (C3, C6- and C10-) of 1-(orto-tolyl)biguanide was synthesized and characterized in terms of surface tension, aggregation properties and antibacterialactivity. Specifically, biguanide derivatives as chloride salts (1-tolyl-5-propyl-biguanide, TBC3; 1-tolyl-5- esyl-biguanide, TBC6; and 1-tolyl-5-decyl-biguanide TBC10) were obtained from a synthetic approach, starting form o-toluidine and sodium dicyanamide in anacid environment. Thermal analysis has revealed that both degradation and melting temperature of the synthesized biguanide derivatives were strongly dependent from the length of the alkyl chain. Specifically, the degradation temperature increased for the TBC10 (334 °C) in comparison to the other derivatives with a shorter alkyl chain (TBC3 and TBC6, degradation temperature in the range 296-326 °C). On the other side, an inverse relationship wasfound between the length of the alkyl chain and the thermal parameter associated to the melting process (melting temperature, Tm and melting enthalpy ΔH). Indeed, the calculated Tm values decreased from 218 °C to 187 °C and ΔH values from 126 J/g to 46 J/g moving from TBC3 to TBC10. As regard surface tension measurements, both the calculated critical micelle concentration (CMC) values and the surface tension at CMC (γCMC), were again dependent on the length of alkyl chain. Similar to other surfactant homologue series, CMC values decreased from 18.9 mN/m to 3.3 mN/m over the elongation of the alkyl chain. However, in the case of the homologues seriesof biguanide derivatives, γCMC values resulted to be also much affected from the length of the alkyl chain,so that only for TBC6 (γCMC ~40 mN/m) and TBC10 (γCMC ~28 mN/m), surface tension dropped below 50 mN/m, indicating that only these two compounds can be considered "effective" surface active molecules. On the other side, for TBC3 derivative, the minimum surface tension value was close to 60 mN/m, indicatingthe lower air-water surface activity of this compound. CMC values were confirmed by conductivity and agood agreement was found between CMC values obtained from conductivity measurements and thosefrom tensiometric analysis. The commercial non-alkylated compound 1-(o-tolyl)biguanide was also analysed for comparison. The MIC values for all biguanide derivatives in comparison to chlorhexidine digluconate were calculated. All compounds showed a better activity against the gram positive *S. aureus* ATCC 6538 than the gram negative *P. aeruginosa* ATCC 15442. TBC10 had the lowest MIC values, despite being more than ten times higher than those calculated for chlorhexidine as reference. The compounds TBC10 and TBC6 passed the disinfectant test (according to UNI EN 1040:2005 and ISO 20776- 1:2019) at the concentration of 0.5% w/w against *P. aeruginosa* and *S. aureus*, since they caused more than 5-times CFU counts Log reduction after 2-min-contact at 20°C. The most effective compound was TBC10 (C10-alkyl chain), highlighting the relevant effect exerted by the length of the alkyl chain both on surface tension, aggregation and antimicrobial/disinfectant activity. According to the obtained results, these biguanide derivatives can be proposed as novel surfactants endowed with antimicrobial and disinfectant activity, potentially employed in pharmaceutical and cosmetic formulations. Specifically, they can find applications in the preparation of biphasic systems with the dual function of stabilizers and preservatives or in the preparation of aqueous formulations for disinfection.

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HOST-DIRECTED STRATEGIES AND SUSTAINABLE - GREEN TECHNOLOGIES TO FIGHT INFECTIOUS AND INFLAMMATORY DISEASES

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The development of alternative and sustainable strategies for infections and inflammatory disorders has always been at the basis of our main research lines.

In line with this purpose, we recently developed host directed strategies to prevent bacteria resistance and target inflammation at the host-pathogen interface. In this regard, we fabricated oral and pulmonary delivery platforms to target epithelial barriers and specific microbial resistance mechanisms and inflammatory pathways. By this approach, 3-indole-aldehyde, was delivered to the lungs and the small intestine to target AhR dependent homeostasis. This compound belongs to a class of microbiota derived tryptophan metabolites that show AhR agonism. 3-IAld proved remarkable activity on immune dysreactive disorders [1] as well as infectious and inflammatory diseases by playing around the pathogen-host crosstalk.

This led to a series of applications targeting a wide spectrum of pathologies [2-4], including serious and life-threatening diseases, such as cystic fibrosis (CF) [5]. Likewise, we also developed a repurposing strategy to treat inflammation in CF. Anakinra, a recombinant homologue of the endogenous IL-1 receptor antagonist and active principle of Kineret®, was directed as a dry powder to the lungs with amazing results in terms of efficacy and safety in infected CF mice compared to systemic delivery [6]. These results encouraged to move towards a promising oral formulation of anakinra that is currently under intensive development for repurposing in CF.

To rescue inflamed and/or infected lung homeostasis, we also proposed an inhalable N-acetylcysteine (NAC) dry powder formulation to potentially replace the commercial solution for nebulization. Besides its antioxidant and immunomodulatory properties, NAC attracted attention as adjuvant therapy in several viral infections. The NAC dry powder showed higher lung deposition, with respect to the nebulized solution, with no need for storage at low temperature [7].

One of the approaches to fight bacteria resistance consists in blocking microbial reflux pumps. Therefore, we formulated newly synthesized indolic inhibitors as inhalable dry powders with leucine and chitosan to halt *Staphylococcus aureus* biofilm formation while ensuring lung cell safety [8].

Pursuing sustainability, we also focused our attention on green approaches and renewable materials to fight infection and inflammation. Following the action track 6 of the One Health Joint Plan of Action 2022-2026 whose aim is "Protect and restore biodiversity, prevent the degradation of ecosystems and the wider environment to jointly support the health of people, animals, plants and ecosystems underpinning sustainable development", we exploited by-product waste, deriving from the food chain, for health purposes. These wastes are generally produced in large quantities and, in some cases, difficult to dispose often requiring high costs. Many studies [9] highlighted that these materials are still useful as a valuable source of bioactive molecules as well as biopolymers. This is very interesting in order to reduce the depletion of natural sources suggesting "smart" solutions for waste disposal by their reuse.

In this scenario we focused our attention on some abundant food wastes as onion skins [10], hazelnutshells [11], *Crocus sativus* petals [12]. Dry extracts were prepared from these wastes by means of green extraction methods using a hydroalcoholic solutions as extraction solvent.

The onion skin dry extract, rich in cyanidin-3-O-(60-malonyl-glycoside) and quercetin, showed good antioxidant and anti-inflammatory activities. Moreover, it demonstrated to be active against *S. epidermidis* and *S. aureus*, bacteria often involved in wound infections. For this reason, this extract was successfully formulated in bioadhesive patches for skin application.

Hazelnut shells are an interesting source of polyphenols useful in health field. Different extraction methods were investigated evaluate their effect on the final chemical composition. It was observed that the main compound extracted resulted gallic acid, a molecule useful for many applications in health due to the antioxidant and anti-inflammatory activities.

Crocus sativus petals are an abundant waste deriving from stigmas harvesting (to obtain 1 kg of spice 110,000-160,000 of petals are produced). The extract obtained, rich in gallic and chlorogenic acids, demonstrated antioxidant activity and ability to stimulate keratinocytes growth in a safe concentration range (0.02-0.4 mg/mL) resulting useful to be applied on damaged skin for healing improvement.

The extract was thus formulated in a corn-starch hydrogel showing both suitable rheological properties and spreadability, necessary for an easy and pain free application on damaged skin. Moreover, in vitro microbiological studies demonstrated a self-preserving property that allow avoid the use of preservatives in the formulation or reduce considerably their use.

In the context of the above-mentioned action track 6, attention has been focused to bees and their products as well. In fact, beekeeping is an example of a circular economy with low environmental impact, it can be said that nothing is more circular than a bee: everything it produces is reused without producing wastes and its role in the ecosystem is universally recognized. Bees supply precious products such as propolis, widely used in traditional medicine for its antibacterial, antioxidative, and anti-inflammatory properties. Therefore, it was proposed in the treatment of wound microbial colonization that is one of the causes of the healing delay or failure. A wound dressing composed of propolis, crustacean waste-derived chitosan and glycerine was prepared by casting to control micro-organisms growth within a wound environment, thus promoting wound healing. The obtained films were prepared by casting and they showed good hydration properties and antimicrobial and antibiofilm activities against Gram-positive bacteria (*S. aureus*, *S. epidermidis* and *P. aeruginosa*) and the *Candida albicans*, low cytotoxicity against fibroblasts, good antioxidant properties and regenerative effect in human fibroblasts by in vitro cell migration assay on human dermal [13].

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NEW HORIZONS FOR DRUG DELIVERY TO THE AIRWAYS

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Biopharmanet TEC

Introduction

Nasal and pulmonary administrations is often associated with products for the management of ailments such as nasal congestion, common cold symptoms, seasonal allergies or more persistent conditions such as chronic sinusitis for the nose or asthma and COPD for the lung.

Nevertheless, the upper and lower airways provide access to a highly vascularized and permeable mucosa, making them attractive not only for topical treatment of local diseases but also for systemic drug delivery. In this respect, the pulmonary administration of insulin has represented a technological milestone, and an excipient-free insulin powder was developed and patented at University of Parma [1]. Moreover, the presence in the airways of immunocompetent cells and the direct access to the brain via the olfactory region are allowing for needle-free vaccination or central nervous system (CNS) targeting. These potential applications, however, have to tackle a number of hurdles related to the physiological and anatomical barriers, such as the presence of a mucus layer, various clearance mechanisms and the necessity of specific deposition patterns in the airways. Hence, the application of advanced formulation strategies and, ideally, novel delivery devices appear pivotal to overcome the notorious limitations of nasal and pulmonary delivery [2]. In the present communication, the potential of airways delivery in the field of CNS diseases, mucosal immunization and local protection and treatment against infections will be showcased through the results of selected research projects carried out also in the framework of several international collaborations.

Nose-to-brain Delivery

Scientific evidences increasingly support the idea that intranasal drug delivery enables brain delivery of both low and high MW APIs actually bypassing the BBB via the innervation of the nasal cavity, *i.e.* the olfactory and trigeminal nerves. In particular, the olfactory 'neuroepithelium', a unique feature of the nasal cavity, directly connects the CNS with the external environment. Simvastatin-loaded nanoparticles were developed in order to exploit the notorious pleiotropic effects of statins (anti-inflammatory, anti-oxidant, immuno-modulating, anti-cancer actions) [3]. In reason of some specific features, such as mucoadhesion due to chitosan coating, prompt biodegradability in nasal environment and rapid drug release and permeation across *in vitro* and *ex vivo* nasal mucosa models, these particles were studied for their application in the treatment of neurodegenerative disease models and for the treatment of brain tumours. In the first case, simvastatin-loaded lecithin/chitosan nanoparticles were shown to regulate cytokines release and protect astrocytes from damage induced by the neurotoxic compound psychosine. Furthermore, the use of simvastatin-loaded nanoparticles inhibited psychosine- induced demyelination in organotypic murine cerebellar slices culture [3]. In another experiment, simvastatin-loaded chitosan-coated lipid-core PCL nanocapsules were demonstrated *in vivo* to be effective against an orthotopic model of glioma in rats. The administration of the nanocapsules for 14 days resulted in a significant reduction (78%) of brain tumor not attainable using simvastatin alone [4].

Mucosal Vaccination

Airways are the port of entry of several pathogens and are physiologically designed to respond to such threats via the mucosa-associated lymphoid tissue (MALT). This opens the possibility of performing mucosal vaccination by intranasal and pulmonary route. Through a multi-component excipient and spray-drying approach, we engineered highly respirable dry-powder vaccine particles containing a three-fold repeated peptide epitope derived from human papillomavirus (HPV16) minor capsid protein L2 displayed on *Pyrococcus furious* thioredoxin as antigen. A key feature of our engineering approach was the use of the amphiphilic endotoxin derivative glucopyranosyl lipid A (GLA) as both a coating agent, enhancing particle de-aggregation and respirability, as well as a built-in immune-adjuvant. Following an extensive characterization of the *in vitro* aerodynamic performance, lung deposition was verified *in vivo* by intratracheal administration in mice of a vaccine powder containing a fluorescently labelled derivative of the antigen (Fig. 1).

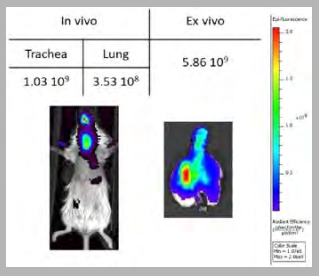

Fig. 1. In vivo and ex vivo, IVIS analysis of Alexa Fluor 750-labeled PfTrx-HPV-L2 accumulation in the respiratory tract, 15 min after intratracheal delivery of the dry-powder vaccine. Reproduced from [5].

This was followed by a short-term immunization study that highlighted the ability of the GLA-adjuvanted vaccine powder to induce an anti-L2 systemic immune response comparable or superior to that of the subcutaneously administered, as the intratracheally administered dry-powder, but not the control vaccine induced consistent HPV neutralizing responses [4].
Similarly, a novel nasal dry powder vaccine was obtained by the layering of a nanoemulsion-based nanoadjuvant and an inactivated form of *M.hyopenumoniae* onto solid pharmaceutical excipients such as mannitol or calcium carbonate. This dry powder vaccine was recently demonstrated in piglets to elicit immune cells recruitment in the nasal tissue within one week from the vaccination and to provide an immune response non-inferior to an intramuscular immunization in terms of *M.hyopenumoniae* specific interferon-γ, IgA and IgG in peripheral blood mononuclear cells [5].

Infection Prevention/Protection/Treatment
Nowadays, pulmonary delivery of antibiotics directly to the airways is accepted as a clinically important approach for the management of lung infections. DPIs have been proposed and identified as promising products for this application due high dose delivery capability and optimal patient compliance.
Our group applied QbD approaches to develop respirable aminoglycoside microparticles containing minimal amounts of excipient for the management of CF infections. The addition of lubricants and the role of ethanol in particle engineering were deeply assessed [6]. Furthermore, the product development focused including into high-dose delivery devices the entire dose of tobramycin powder and *in vivo* data were collected as well to assess the dose delivered according to the inhalation profiles [7].
Recent data suggest a correlation between altered lung microbiota (*i.e.* dysbiosis) and respiratory diseases, including cystic fibrosis (CF). An innovative approach based on the inhalation of probiotics was investigated by our group and preliminary results strongly suggest a potential role of these microorganisms in managing CF respiratory infections. Spray-dried powers of *L.rhamnosus*, *L.acidophilus* and *L.plantarum* were developed to maximise respirability and probiotics viability. *L.plantarum* was shown to remarkably interfere with the growth of *Pseudomonas Aeruginosa* (Fig. 2).

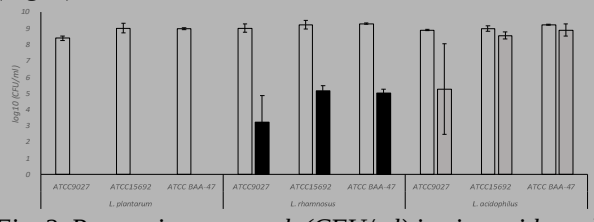

Fig. 2. P.aeruginosa growth (CFU/ml) in vitro with and without lactobacilli loaded in inhalable dry powders.

To increase the efficacy of a pulmonary treatment of mycobacterial infections an inhalable antibiotic powder targeting infected alveolar macrophages (AMs) and including an efflux pump inhibitor was developed. Low molecular weight sodium hyaluronate sub-micron particles were efficiently loaded with rifampicin, isoniazid and verapamil, and transformed in highly respirable microparticles by spray drying, providing a slow-release, favourable to maintain a high drug level inside AMs. *In vitro* antimicrobial activity and *ex vivo* macrophage infection assays evidenced more than 80% reduction in bacterial viability irrespective of the drug resistance profile [8]. Furthermore, antimicrobial peptides (AMP) containing inhalable powders are under development. AMP display a broad-spectrum activity against gram-positive and gram-negative bacteria, mycobacteria and fungi and can be exploited in the modulation of the immune system response.
The recent COVID-19 pandemic has led to the development of several products not only for the mucosal vaccination against the virus but also for the local treatment or prevention of the viral infection. Nasal and pulmonary formulations of cyclosporine A were developed and tested *in vitro* to evaluate its potential use as a prevention or treatment of early infections with SARS-CoV-2. Micelles and easily dissolving dry powders were developed for nasal and pulmonary administration, respectively. The formulations evidenced an excellent antiviral effect in an *in vitro* model of infection carried out on Vero E6 cells using Omicron BA.1 viral variant. In particular, it was demonstrated that TPGS micelles acted synergistically with the drug to reduce viral burden and cell mortality. The formulation of hyperimmune serum against SARS-CoV-2 as dry powder preserved the neutralizing ability of IgGs after spray drying. Aerodynamic performance of powders was assessed *in vitro*, showing high deposition in the nose and reaching the deep airways too, potentially providing a broad coverage against different virus variants.

Conclusions
Nasal and pulmonary delivery appears to offer an interesting alternative to more invasive administration routes for impactful treatments aimed at CNS disease, emerging infections, not to mention peptides, proteins, and nucleic acid delivery. However, the development of a next generation of nasal products requires the application of new formulative approaches, delivery devices and characterization tools.

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THE ROLE OF ITALIAN COMMUNITY PHARMACY IN PRIMARY SECONDARY AND TERTIARY PREVENTION: TEN YEARS OF EXPERIENCE.

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Università degli Studi di Torino, Dipartimento di Scienza e Tecnologia del Farmaco

Sociodemographic changes in the Italian and European population highlight the need for new public health management, particularly regarding diseases prevention and adherence to therapy. In this context, Federfarma Piemonte, in concert with the Order of Turin Pharmacists and the University of Turin, developed a Project named "Community Pharmacy" whose aim is to enhance the pharmacist's role in public health.

The Project is based on a continuum evaluation of the new community pharmacist's role through the carrying out of specific studies. The Project involves a first planning phase to evaluate the study feasibility, in collaboration with experts in the field. A construction phase then follows, which initially involves univocal pharmacists training and then the experimental activity in pharmacy. The experimental activity usually consists of the engagement of subjects with specific inclusion criteria and in a univocal pharmacist's intervention in all the pharmacies involved in the project. Finally, the evaluation phase consists of the analysis of the results coming from the pharmacist's activity in order to perform an impact assessment.

Following this scheme, we investigated principal chronic diseases (es. hypertension, diabetes, heart failure, chronic obstructive pulmonary disease (COPD), asthma, migraine) through the administration of questionnaires and/or the use of specific instruments and finally we performed an epidemiological evaluation of gathered data.

Results are encouraging and in accordance with the regulatory framework.

Topics

Disease prevention

The prevention of chronic and acute diseases brings to a reduction in the incidence of these diseases and also significant cost savings for the National Health Service (NHS). In this context, community pharmacists, properly trained, can play as an epidemiological sentinel: they can disseminate healthy lifestyles, they can identify those who are most at risk of developing a specific disease and patients unaware of their status. Then, they can perform an adequate counselling and/or address the subject to the most appropriate medical facility. Community pharmacists can use questionnaires, test instruments and telemedicine tools.

Adherence to therapy

A pharmacological treatment requires the patient to take the medicines as prescribed by the practitioner to be effective. An insufficient adherence to therapy can compromise the efficacy of the treatment, causing a risk for patient's health and a waste of NHS resources. In this context, an adequately trained community pharmacist, can contribute by monitoring the intake of the medicines and by giving necessary advice to enhance therapy compliance.

Methods

Questionnaires specially drawn up in collaboration with medical specialists, and/or internationally validated, for the assessment of primary secondary or tertiary prevention.

Test instruments, telemedicine tools, online platform for data collection,

Statistical software to analyze the results

Collaborations

- Pharmacy Owners Association "Federfarma"
- Orders of Pharmacists
- Regional Departments
- Medical specialists

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Tuesday, September 12

Chairperson *Paolo Caliceti*

9.00-9.40 Prof. Dan Peer, University of Tel Aviv
Play God with RNA

Chairperson *Ruggero Bettini*

9.40-10.20 Prof. Stefaan De Smedt, University of Ghent
Nucleic Acid Delivery: on recent findings from the Ghent Research group on Nanomedicines

10.20-10.50 *Coffee break*

PLENARY LECTURE

RNA Vaccines and Therapeutics: from Gene Silencing to Genome Editing

Dan Peer
Director, Laboratory of Precision NanoMedicine
Tel Aviv University
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Accumulating work points out relevant genes and signaling pathways hampered in human disorders as potential candidates for therapeutics**.** Developing nucleic acid-based tools to manipulate gene expression, such as siRNAs, mRNA and genome editing strategies, open up opportunities for personalized medicine. Yet, although major progress was achieved in developing RNA targeted delivery carriers, mainly by utilizing monoclonal antibodies (mAbs) for targeting, their clinical translation has not occurred. In part because of massive development and production requirements and high batch-to-batch variability of current technologies, which relies on chemical conjugation. Here we present a self-assembled modular platform that enables to construct theoretically unlimited repertoire of RNA targeted carriers. The platform self-assembly is based on a membrane-anchored lipoprotein, incorporated into RNA-loaded novel, unique lipid nanoparticles that interact with the antibody Fc domain. We show that a simple switch of 8 different mAbs, redirects specific uptake of siRNAs by diverse leukocyte subsets *in vivo.* The platform therapeutic potential is demonstrated in an inflammatory bowel disease model, by targeting colon macrophages to reduce inflammatory symptoms, and in Mantle Cell Lymphoma xenograft model, by targeting cancer cells to induce cell death and improve survival. In addition, I will discuss novel approach for delivering modified mRNA to specific cell types *in vivo* utilizing this platform. I will also share some data on the first mRNA vaccine for extracellar bacterial infection. Finally, I will share new data showing very high efficiency genome editing in glioma, metastatic ovarian cancer and mantle cell lymphoma. I will include several translational stories going from the lab to clinical trials in this field with challenges and opportunities.

Dan Peer is a Professor and the Director of the Laboratory of Precision NanoMedicine at Tel Aviv University (TAU). He is also the Vice President for Research and Development at Tel Aviv University. From 2017 - Present, he is the Founding and Managing Director of the SPARK program of Translational Medicine at TAU.

PLENARY LECTURE

Nucleic Acid Delivery: on recent findings from the Ghent Research group on Nano-medicines

Stefaan C. De Smedt

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In the last decade, life science research has focused on novel biotechnological drug candidates with activity directed against intracellular targets instead of targets located on the cell-surface. A few examples are intracellular enzymes (e.g. for the treatment of lysosomal storage disorders), monoclonal antibodies, mRNA (e.g. cancer vaccines) and plasmid DNA (gene therapy). Also, interfering RNAs (siRNA, miRNA) emerged as interesting molecules with the potential to suppress the expression of any desired gene, making them usable to treat a broad range of (currently incurable) diseases. In contrast to current (small molecule) drugs, these (large-molecule) biotech-compounds can not spontaneously diffuse across cellular membranes. Academics, as well as small and big farma companies are searching intensively for technological solutions to deliver such biotech-compounds inside target cells in vivo. Indeed, the lack of safe and advanced delivery-technologies is considered as one of the major bottlenecks for the ultimate breakthrough of many of these biotech-compounds.

One approach to deliver biotechnological compounds intracellularly is by encapsulation in nano-sized particles (nanomedicines). Meanwhile it is generally accepted that after uptake by cells, such nanomedicines typically end up in endo-lysosomal vesicles in which they remain entrapped while they should escape from such compartments and arrive in the cytosolic fluids of the cells. In recent years our team undertook major efforts to understand the biophysics which play a role in (a lack of) escape of nanomaterials from endo-lysosomal vesicles. Vere recently we discovered new chemical strategies (so named 'escape adjuvants') which seem promising to 'liberate' nucleic acids (like siRNA) from endo-lysosomal vesicles into the cytosol. A first strategy uses cationic amphiphilic drugs (CADs), being medication which is daily taken by numerous patients for various indications. CADs trigger transiently and non-lethally lysosomal membrane permeabilization thereby enhancing cytosolic delivery of lysosomal sequestered siRNA. A second strategy exploits surfactant protein B (SP-B), an essential component of pulmonary surfactant; Indeed, rather unexpected we discovered that SP-B shows the potential to promote siRNA delivery from endo-lysosomal vesicles into the cytosol of various cell types, including lung epithelial cells and alveolar macrophages.

In recent years we also explored physical methods which can 'mildly' deform cellular membranes and which show potential to directly deliver bio-therapeutics into the cytosol, thereby bypassing the endo-lysosomal routes. Besides our long standing interest in the use of ultrasound waves for transfecting cells we got a recent major interest in the use of light to deliver biologically active compounds. In our hands, so named 'photoporation', especially in combination with gold nanoparticles, seems promising for efficient in vitro intracellular delivery of macromolecular delivery in live cells.

This lecture will explain our recent efforts in finding strategies to deliver biotechnological compounds, especially nucleic acids, from the extracellular space, over cell- and organelle-membranes, into the cytosol. Both pharmaceutical, biological and engineering aspects of our work will be highlighted.

Professor **De Smedt** graduated from Ghent University (Belgium) in 1995 and joined the pharmaceutical development group of Janssen Research Foundation. In 1999 he became Professor in Physical Pharmacy and Biopharmacy at Ghent University where he initiated research on advanced delivery of biologics/nanomedicines and founded the Ghent Research Group on Nanomedicines. The research focus in his lab is on the delivery of bio-therapeutics, nucleic acids and proteins, for future therapies of lung and ocular diseases and cancer (through mRNA vaccination and cell therapies).

Professor De Smedt served as dean of his faculty between 2010 and 2014. From 2014 till 2022 he has been a member of the Board of Directors of respectively Ghent University and the Academic Hospital of UGent. He has been a Guest Professor at various universities in Belgium and China. Since 2004 he serves as the European Editor of the Journal of Controlled Release; since 2023 he leads the JCR as Editor-in-Chief. He is a Distinguished Visiting Scientist of the Chinese Academy of Sciences. He has been elected as member of the Flemish Royal Academy of Medicine, the European Academy of Sciences and the Académie Nationale de Pharmacie de France.

Tuesday, September 12
Session 3

Room A - Sala Tergeste

Chairpersons *Paolo Blasi - Ida Genta*

10.50-11.15 Multicargo polymeric nanoparticles to overcome resistance in cancer chemotherapy

Fabiana Quaglia, University of Naples Federico II

11.15-11.40 Core and surface engineering of "smart" drug nanocarriers

Stefano Salmaso, University of Padova

11.40-12.05 Less is more. From liposomes to nanocrystals.

Chiara Sinico, University of Cagliari

12.05-12.30 Zein as a versatile biomaterial: new perspectives for innovative applications

Donato Cosco, University Magna Graecia of Catanzaro

12.30-14.30 Lunch

MULTICARGO POLYMERIC NANOPARTICLES TO OVERCOME RESISTANCE IN CANCER CHEMOTHERAPY

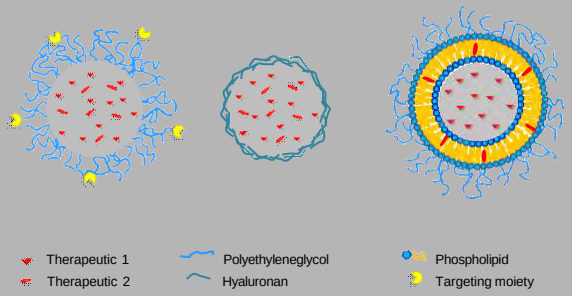
G. Longobardi, C. Conte, F. Quaglia

Università degli Studi di Napoli Federico II, Dipartimento di Farmacia

The acquisition of resistance to chemotherapy is a major hurdle in the successful application of cancer therapy. Chemotherapeutics either lose efficacy in a short time during/after the treatment or have no efficacy at all at the currently employed dose regimen. Switching to other class of chemotherapeutics often does not provide a solution due to the rapid induction of multidrug resistance (MDR) in cancer cells [1]. For this reason, the strategy against cancer needs to shift from finding new therapies to improving existing therapies in innovative and effective ways.

The delivery of multiple therapeutics acting on different cellular targets has been foreseen as a viable solution. However, it remains a pharmacological challenge to design combination therapies in which the components work synergistically. In fact, each drug has its own PKs and it is hard to realize a precise combinatory dose in the target tissue [2]. To this purpose, nano-based co-delivery can be advantageous to control the biodistribution of the combination in the body, attain accumulation, internalization, and release of the cargo payload in the target cell at appropriate ratios. Furthermore, nanoparticles (NPs) can help the overcoming formulation shortcomings of some chemotherapeutics, such as low solubility in pharmaceutical vehicles for injection. The liposomal combination Vyxeos® (cytarabine/ daunorubicin 5:1) is the first example of precise delivery via nanotechnologies.

In this context, we have been focused on developing different panels of polymeric NPs delivering multiple therapeutics (Figure 1). NPs were selected since they offer the unique advantage to manipulate their features by playing on the chemistry and arrangement of the structural components. This flexibility allows control of the drug payload location inside NPs, its release rate, and the chemical identity of the nanoplatform.



In the first research direction, we have exploited a new therapeutic concept relying on the combination of chemotherapy with light-activated treatment modalities supported by a nanotechnological approach. As to "unconventional" approaches, our interest goes to treatment modalities that point at therapeutic agents such as Radical Oxygen Species and Nitrogen Oxygen Species (ROS and RNS, respectively) that can be produced in the confined environment of tumors in a controlled way. ROS trigger necrosis and apoptosis of cancer cells, immune stimulation, and shutdown of microvessels, finally inducing tumor regression [3]. Amid RNS, nitric oxide (NO) is a multitarget molecule that acts as cytotoxic species itself and overcomes MDR mechanisms also in the hypoxic conditions typical of the tumor environment [4]. In the last few years, we have developed a panel of biodegradable NPs delivering combinations of chemotherapeutics currently employed in the clinic and existing/novel agents generating singlet oxygen and/or NO under the input of light. Here, the technological challenge is to avoid intermolecular processes due to the confinements of the therapeutic components within the restricted space of the NPs that may, in principle, preclude the correct functioning of the final nanoassembly. We are now investigating how photoactivatable NO-releasing NPs overcome intrinsic and acquired resistance mechanisms in different 2D and 3D cancer cell lines.

In a second research direction, we have exploited combinations to overcome resistance to 5-Fluoruracil (5-FU) in colon cancer. 5-FU was combined with i) β-carotene, which can reduce the expression of the ATP-binding cassette transporters responsible for the drug efflux [5]; ii) novel peptides (VLP-13 and VLP-24) inhibiting the MDM2/4 heterodimer, i.e. the main responsible of p53 suppression and resistance mechanisms. To this purpose, we have designed polymeric NPs with a core of poly(lactic-co-glycolic) acid entrapping the drug and a shell of hyaluronan (HA) targeting CD44 receptor overexpressed in colon cancer cells. In both cases, a therapeutic PoC was provided in vitro.

Finally, we are developing core-shell NPs to deliver combinations of chemotherapeutics and nucleic acids to silence specific proteins or express defective proteins involved in MDR mechanisms. The NPs are based on biodegradable polyesters and amine-bearing components to incorporate both a lipophilic chemotherapeutic and a hydrophilic negatively-charged

the context of lung cancer. We are currently trying to engineer the NPs to target different organs and cell populations thus enlarging the benefit of this delivery technology.

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CORE AND SURFACE ENGINEERING OF "SMART" DRUG NANOCARRIERS

S. Salmaso, B. Arpac, R. Daniele, L. Marcenta, G. Bellio, F. Tognetti, C. Pesce, A. Grigoletto, M. Garofalo, F. Mastrotto, G. Pasut, P. Caliceti

Department of Pharmaceutical and Pharmacological Sciences, University of Padova, via F. Marzolo 5, 35131 Padova-Italy

The biopharmaceutical properties of nanocarriers for delivery of therapeutics stem from the rational combination of components dictating the supramolecular properties. The core and the surface features of carriers assembled according to a bottom- up-process require fine design of the components and the strategies of assembly.
These systems have been conceptualized for the treatment of few diseases where local microenvironment can be exploited to control the interaction with the biological interfaces, the drug release, the clearance, and the response to remote activation by external physical stimuli.

"Smart" core engineering

Polyaminoacid based self-assembly micelles were generated with rationally designed aminoacid composition for the delivery and controlled release of Doxorubin to cancer. A set of random co-polymers (mPEG-r-[(Glu-hydrazine)$_m$-(Leu)$_n$]) were synthesized by Ring Opening Polymerization from 5 kDa mPEG-NH_2 macroinitiator using 16:0:1, 8:8:1, 6:10:1, 4:12:1 γ-benzyl glutamic acid carboxy anhydride monomer/leucine N-carboxy anhydride monomer/PEG molar ratios, mPEG-Glu/Leu random co-polymers were generated. The benzyl ester protecting the γ- carboxyl group of glutamic acid was quantitatively displaced with hydrazine to yield mPEG$_{5kDa}$-b- (hydGlu$_m$-r-Leu$_n$). Doxorubicin was conjugated to the diblock co-polymers through pH-sensitive hydrazone bond to Glu-hydrazine for pH-controlled intracellular drug release. The Glu-/Leu ratio of the mPEG-r- [(hydGlu)$_m$-r-Leu$_n$] library results in different conjugation efficiencies of Doxorubicin; the distancing of Glu promoted the conjugation of Doxorubicin. The conjugation of Doxo and the increase of Leu/Glu enhanced the self-assembly according to the CLM that was below 5 μM for the mPEG$_{5kDa}$-b-[(Doxo-hydGlu)$_6$- *r*-Leu$_{10}$]. At pH 5.5, mimicking endosome environment, the carriers containing leucine showed a faster Doxo release than at pH 7.4, mimicking the blood conditions. Doxo-loaded colloidal formulations showed a dose dependent cytotoxicity on two cancer cell lines, CT26 murine colorectal carcinoma and 4T1 murine mammary carcinoma with IC_{50} slightly higher than those of free Doxo. The cytotoxicity correlated with the Leu content which was found to facilitate the release. Confocal imaging on CT26 cell line showed that intracellular fate of the carrier involves a lysosomal trafficking pathway. The intratumor or intravenous injection to CT26 and 4T1 subcutaneous tumor bearing mice yielded higher antitumor activity compared to free Doxo. Furthermore, mPEG$_{5kDa}$-b-[(Doxo-hydGlu)$_6$-r-Leu$_{10}$] displayed a better safety profile when compared to commercially available Caelyx®. [1]

A lipid nanoplatform was developed for the oral delivery of the therapeutic peptide exenatide. Peptides, because of their hydrophilic properties, are difficult to be encapsulated into a hydrophobic lipid matrix [2], which on the other hand offers the benefit of erosive release with respect to polymeric carriers and no accumulation of matrix component in the body. The core of the lipid particles was engineered to enhance peptide loading efficiency. This platform is based on solid lipid nanoparticles that have been assembled by microfluidic process of phospholipid/cholesterol in ethanol and the aqueous phase containing exenatide pre-complexed with a cationic lipid.
Preliminary studies with exenatide-free SLN were performed to identify the phospholipid/cholesterol molar ratio and concentration, and the microfluidic conditions yielding small homogeneous SLN. Exenatide was pre-complexed at increasing cationic lipid/peptide molar ratios on the 0:1-18:1 range and each set of ratios was processed at increasing exenatide/total lipid feed ratio (5-15% w/w). 3:1 flow rate ratio and 12 mL/min total flow rate were selected to generate the exenatide-loaded SLN since these conditions resulted in small particles and low dilution of the samples during assembly.
The peptide loading efficiency in SLN without pre-complexation with the cationic lipid was low (< 10%) while increasing up to 90% with a 12:1: cationic lipid /peptide molar ratio for all the peptide/total lipid feed ratios tested. However, peptide/total lipid feed ratio above 10% resulted in particles aggregation, thus 12:1: cationic lipid /peptide molar ratio and 10% peptide/total lipid feed ration were selected since they compromise high loading efficiency and size below 150 nm.
Extensive colloidal characterization of SLN was performed. Particles had a zeta potential of 50 mV due to excess of cationic lipid. TEM analysis of peptide-loaded SLNs showed particles with spherical shape and size in agreement with that detected by DLS (~120 nm). The colloidal stability of SLNs in simulated intestinal fluid (SIF, pH 6.8) and PBS (pH 7.4) were remarkable up to 48 hours. However, SLN aggregated quickly in the presence of FBS.
Release in simulated SIF, pH 6.8 and in PBS, pH 7.4 showed an initial faster release in the first 10 hours,

followed by a prolonged and sustained release afterwards with complete peptide release in 14 and 22 days, respectively. The preservation of the conformational and chemical stabilities of loaded exenatide were investigated by circular dichroism (CD) and ESI-TOF, upon release. Two formulations at different PEG coating densities were generated by incubating SLN with DSPE-PEG_{2kDa} and zeta potential analysis was used to confirm surface charge shielding. The PEG density on particle surface generated particles with neutral zeta potential at 30 % DSPE-PEG/lipid w/w ratio. Finally, with the perspective of administering the SLNs by enteric-coated capsules, were lyophilized with 1:1 w/w mixtures of two lyoprotectants, mannitol and trehalose, at increasing concentrations (1-2% w/v) and the stabilizing efficiency was derived from the size after SLN redispersion. 2% (w/v) trehalose/mannitol resulted as the lowest lyoprotectant concentration providing the recovery of particle size after redispersion in water. SLN were loaded in enteric-coated capsules, that showed high integrity for 2 h in simulating gastric conditions (pH 2), while in simulated intestinal fluid (pH 6.8) disintegration of the capsule and quantitative release of particles was observed in 1 hour.

"Smart" surface engineering.

Liposomes have been investigated by engineering their surface properties in order to modulate the interfacing ability with the biological environment. Focus was given for the intravitreal administration of drug in age related macular degeneration treatment affecting the posterior segment associated ocular diseases [3]. Delivering therapeutic molecules to the eye has always been a challenge because of the involvement of various anatomical and physiological barriers, e.g the blood retinal barrier (BRB) [4]. Surface of liposomes was engineered to control either their diffusivity in the vitreous and their intracellular access across RPE.
Deaxamethasone loaded Liposomes were prepared using HSPC/Cholesterol molar ratio by a remote loading process. A variety of formulation parameters were investigated to assess the effect on the loading efficiency and capacity: medium pH, phospholipid to drug ratio, incubation time and temperature. Liposomes were decorated with 5 mol% of a newly synthesized cell penetration enhancer (CPE), or 5% $mPEG_{2\ kDa}$- DSPE or 5% CPE/ $mPEG_{2kDa}$-DSPE. Liposome formulations presented a size of ~170 nm and Zeta Potentials (ZP) of +22.4, +3.6 and 0.7 for CPE decorated liposomes (CPE-Lipo), CPE/$mPEG_{2kDa}$- DSPE decorated liposomes (CPE/PEG-Lipo) and $mPEG_{2kDa}$-DSPE coated liposomes (PEG-Lipo), respectively. Dexamethasone hemisuccinate (DH) was remotely loaded into liposomes with a loading capacity of ~10 wt/wt%. The vitreous mobility of these liposomes was tested using Multiple Particle Tracking analysis on explanted porcine eyes. The vitreous diffusion coefficient demonstrated that CPE-Lipo migrate very slowly in the vitreous matrix (diffusivity = 0.004 $\mu m^2/s$) as consequence of the interaction of the cationic surface charges with the anionic charges of hyaluronic acid. PEG-Lipo, on the other hand, are fast moving in the vitreous (diffusivity = 0.174 $\mu m^2/s$), while the liposomes coated simultaneously with CPE and PEG possess intermediate mobility (diffusivity = 0.107 $\mu m^2/s$) resulting from the modulation of the electrostatic interaction of the CPE with the vitreous which can offer a benefit in term of biodistribution and clearance. The formulation containing both CPE and PEG was further investigated demonstrating to be stable in buffer over 20 days. Liposomes with 5 % CPE coating were well tolerated by ARPE-19 retina cells either with or without $mPEG_{2kDa}$-DSPE. Flow cytometric analysis and confocal microscopy demonstrat5ed that CPE promots the association of liposomes to ARPE-19 cells with respect to plain liposomes, while $mPEG_{2kDa}$-DSPE slightly reduced the cell interaction. Interestingly, $mPEG_{2kDa}$-DSPE coating reduced the rate of DH release and enhanced the disposition of CPE coated liposomes in the ARPE-19 cell cytosol resulting in a more efficient anti-inflammatory effect which was tested by IL-6 detection on ARPE-19 cells stimulated with LPS. Finally, $mPEG_{2kDa}$-DSPE enhanced the association of DH- loaded Agm_6-M-Oleate coated liposomes to explanted rat retina, and provide for infiltration of the carrier across all layers of the retina. Additionally, the DH loaded liposomes provide for a significant therapeutic effect on ex-vivo treated retina which reflected in higher viability of inner and outer nuclear layer cells.
The liposomes of this study are expected to offer some benefits over Ozurdex, the intravitreal implant for dexamethasone release [5] since they can be injected with a small needle (30 G) minimize the lens opacification common of corticosteroids, [6] since liposomes do not permeate the lens.

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LESS IS MORE. FROM LIPOSOMES TO NANOCRYSTALS

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The "Technologies for Drug Delivery" research group of the University of Cagliari have been working on the development of liposomal drugs since the early 90s. Since then, our research has been mainly focused on liposomal formulation features, which control the delivery of bioactive molecules into the skin as well as on the mechanisms by which liposome improve skin delivery. In this context, a very promising approach was developed in 2006. In this study, to find the optimal formulation for the topical delivery of tretinoin, the influence of several parameters such as vesicle size and composition, surface charge, and vesicle stability were investigated. Moreover, in the attempt to elucidate liposome–skin interactions, results of the permeation experiments were compared with a visualization study of the treated skin using transmission electron microscopy. The obtained data clearly showed that vesicle size and lamellarity did not affect tretinoin delivery through the skin. In fact, for each composition the permeation profile was very similar for both multi- and unilamellar vesicle dispersions, suggesting that intact penetration of liposomes does not occur. Surprisingly, we obtained higher drug permeation by using gel-state vesicles, while drug retention into the skin was higher with fluid-state liposomes [1]. Overall, the definite mechanism whereby vesicles (trans)dermal drug delivery is achieved is a controversial issue because they can act as penetration enhancers or as carriers. For these reasons, this direction of the research was intensified in early 2000, when our group developed the so-called Penetration Enhancer-containing Vesicles (PEVs), liposomes containing in their composition a penetration enhancer (PE) as carriers for dermal delivery of different drugs, such as minoxidil, diclofenac and tretinoin. During these years, PEVs have been prepared using various PEs, different for physicochemical properties and mechanism of enhancement, with the aim of finding new stable and efficient carriers for skin drug delivery [2]. Overall results obtained during these years have demonstrated that PEVs are especially able to favour drug deposition into the skin layer. Their properties are strongly affected by the used PE and its capability to interact with the liposomal bilayer. Although the literature is rich with promising results obtained using vesicles modified with different additives, the number of products that has been translated into clinical practice is still limited. This is likely the consequence of unresolved problems including the difficulty to guarantee batch-to-batch reproducibility in large-scale productions, costs, procedures, and controls [2].

As known from the literature, about 40% of drugs in the development pipelines and approximately 70% of drugs coming from synthesis or high throughput screening are poorly soluble in aqueous media [3]. With this issue in mind, since 2009, our research activities have been also focused on a simple and promising formulation approach, namely nanocrystals, useful to increase bioavailability of poor water-soluble drugs [4]. Nanocrystals can be defined as nanoparticles of pure drug without any matrix material with an average diameter below 1 µm (typically in the range of 200-500 nm). Usually, they are prepared as a colloidal dispersion of nanocrystals (nanosuspensions) in an aqueous or non-aqueous dispersing phase and stabilized with different surfactants or polymers. The reduction of the mean diameter of the crystals to below a micron determines an increase in both the dissolution rate and the solubility of very poorly water-soluble drugs, resulting in an increased bioavailability. The simplicity of the formulation, its demonstrated efficacy in vivo and the possibility of preparing large volumes at an industrial level have allowed the marketing of a dozen of products based on nanosuspensions. Over the years, we developed nanosuspension preparations of several model drugs intended for different routes of administration (namely dermal, oral, pulmonary, and intraperitoneal, subcutaneous) [5-8]. As expected, all the chosen model drugs exhibited an increased solubility when formulated as nanocrystals. Moreover, with the aim of expanding the delivery methods of nanosuspensions, we demonstrated the feasibility of using them in combination with different devices (Needle-free jet injectors, microneedles, aerosol nebulizer, and e-cigarettes). The different approaches investigated highlight the great versatility of the nanosuspensions as drug delivery strategy to improve drug bioavailability and therapeutic performances. In particular, pulmonary delivery of curcumin and beclomethasone nanosuspension through a classic aerosol nebulizer or an electronic cigarette was studied. The obtained nanosuspensions proved to guarantee an efficient pulmonary delivery when tested with both devices.

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VERSATILE BIOMATERIAL: NEW PERSPECTIVES FOR INNOVATIVE APPLICATIONS

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The impact of natural polymers on the pharmaceutical and biomedical fields is exponentially increasing. In this context, zein, a prolamin-rich protein contained in the endosperm of corn, is one of the most promising biopolymers due to its peculiar and versatile properties, availability, and because it can be obtained from renewable and inexpensive sources. In addition, it is a biomaterial that has been generally recognized as safe by the U.S. Food and Drug Administration thanks to its biocompatibility and biodegradability and because it is characterized by a low degree of toxicity [1]. Our research team recently developed and characterized zein nanoparticles as a function of various parameters, i.e. nature of stabilizers, serum incubation, temperature, pH, as well as cryoprotectants used to obtain suitable freeze-dried systems. Namely, among the emulsifiers used to stabilize the zein matrix, sodium deoxycholate (SD, 1.25% w/v) phospholipon 85G (PL85G, at a ratio of 7:3 w/w with respect to the protein), and Brij O10 (0.2% w/v), promoted the development of nanosystems characterized by a mean diameter of <200 nm and a narrow size distribution [1,2]. SD-stabilized zein nanoparticles were exploited to enhance the antitumor features of sclareol (SCL). The evaluation of the loading capacity of the nanosystems gave a noticeably high value (~12%) when 1 mg/ml of SCL was used, confirming the excellent ability of the natural biopolymer to effectively retain poorly water soluble compounds. Moreover, the nanosystems were characterized by a sustained drug release (up to a week), and this may reduce the number of administrations and consequently the potential side effects. The nanosystems increased the cytotoxicity of SCL with respect to the free compound against various cancer cell lines [3]. Additional investigations demonstrated that it is possible to obtain hybrid nanosystems by mixing zein and PL85G, and that they can be exploited for the protection and delivery of all-*trans*-retinoic acid (ATRA). The encapsulation of the compound promoted an increase of its antitumor features, as a consequence of the cell uptake of the colloidal systems. In addition, they have also demonstrated to provide a great degree of protection of ATRA against UV light-induced degradation with respect to the free form of the active compound, and this effect was due to the peculiar structure of the lipopolymer which contains aromatic and double bond residues able to absorb UV light, avoiding the photochemical degradation of the drug [4]. Moreover, it was demonstrated that it is possible to develop blend nanoparticles by using zein and silk sericin (a hydrophilic animal glycoprotein) as biomaterials, for the delivery of 5-fluorouracil (5FU). The nanoblends showed mean sizes of 100–150 nm, a monodispersed population, excellent physical stability in complex media for up to 12 h as well as up to 6 months of storage. Our results suggest that blend nanoparticles could be safe and useful drug carriers for intravenous administration, offering an attractive new alternative for enhancing the therapeutic efficacy of drugs and it paves the way for potential preclinical applications [5]. Additional studies concerning the gelling properties of zein have been performed in order to investigate the versatility of the protein as biomaterial by means of passive and dynamic rheology. The obtained results showed a strong solid-like feature, a pseudoplastic behavior of the formulations and a good spreadability, suggesting their potential application as topical gels and for food coating/packaging. Furthermore, rutin, a polyphenolic bioflavonoid found in many plants was entrapped into zein gels in order to obtain a formulation for wound healing application. This approach avoided the use of toxic solvents for the administration of the flavone glycoside and the potential adverse effects. *In vitro* scratch assay performed on human keratinocytes showed that the proposed gel formulations promoted the cell migration and a rapid gap closure within 24 h. In addition, the *in vivo* activities of rutin-loaded zein gel showed a greater therapeutic efficacy in Wistar rats than the free form of the active compound and the commercial DuoDERM®, and it significantly decreased several inflammatory cytokines, i.e. TNF-α, IL-1β and IL-6. The biocompatibility, anti-inflammatory activity and suitable morphological and mechanical properties make the zein gels containing rutin promising formulations for wound healing purposes.

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Tuesday, September 12
Session 4

Room B - Sala Zodiaco

Chairpersons *Aurelie Schoubben – Cristina Bonferoni*

10.50-11.15 From scleroglucan to 3D-bioprinting: a 25-year story

Maria Antonietta Casadei, University Sapienza

11.15-11.40 Design and development of drug delivery systems based on swellable hydrophilic polymers

Alessandra Maroni, University of Milan

11.40-12.05 Improving on nature: successfully nanocarriers for the optimisation of biopharmaceutical properties of natural compounds and plant extracts

Maria Camilla Bergonzi, University of Florence

12.05-12.30 Progress of pharmaceutical technological and cosmetic research at Genoa university

Gabriele Caviglioli, University of Genoa

12.30-14.30 Lunch

FROM SCLEROGLUCAN TO 3D-BIOPRINTING: A 25-YEAR STORY

Patrizia Paolicelli, Stefania Petralito, Laura Di Muzio, Vito Cosimo Carriero, Barbara Bigi, Maria Antonietta Casadei

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"Sapienza" University of Rome, Department of Drug Chemistry and Technologies

25 years ago, my experience in the field of pharmaceutical technology and in particular in the fascinating world of polymers began. Professors Alhaique and Coviello suggested me to modify scleroglucan in order to obtain hydrogels suitable for drug release. The synthesis of the carboxymethyl scleroglucan was then optimized and allowed us of obtaining an extremely versatile derivative, able to form physical gels with or without the addition of divalent cations.[1] In fact, while the derivative with a low degree of derivatization allowed to obtain by adding calcium ions thermo-reversible gels with characteristics that depended on the concentration of the starting polymer and of the salt one, [2,3] the derivative with a high degree of derivatization was able to form physical gels only by varying its concentration. These physical gels turned out to be pH-sensitive and suitable for the colon delivery of drugs administered by oral route.[4] Physical hydrogels of high- carboxymethylated scleroglucan were also investigated as potential systems for topical drug delivery using three different therapeutic molecules. Experimental results and theoretical modelling highlighted that, in the absence of drug/polymer interactions (as for fluconazole and betamethasone) the matrix offers negligible resistance to drug diffusion and a Fickian transport model can be adopted to estimate the effective diffusion coefficient in the swollen hydrogel. The presence of weak drug/hydrogel chemical bonds (as for diclofenac), confirmed by rheological tests, slows down the drug release kinetics and a non-Fickian two-phase transport model has to be adopted.[5] During the XVII ADRITELF meeting that was held in October 2000 in Catania, I was introduced to prof. Giammona and my collaboration with his group started. He asked me to synthesize a methacrylic derivative of dextran to combine this derivative with the methacrylate analogue of polyaspartamide, synthesized by his group, in order to obtain new hydrogel structures suitable for the modified drug release.[6] Afterwards methacrylic derivatives of dextran having carboxyl groups at different chain length were synthesized obtaining pH-sensitive hydrogels.[7] These derivatives were also combined with analogous derivatives of polyaspartamide in order to prepare and characterize novel hydrogels with polysaccharide-polyaminoacid structure, able to undergo an enzymatic hydrolysis in the colon and potentially useful for treating inflammatory bowel diseases.[8,9] In the meantime, dr. Paolicelli joint me in the laboratory. Our interest began to turn to the world of tissue engineering and by combining dextran methacrylate and native or derivatized scleroglucan it was possible to create new interpenetrated networks (IPN) with different characteristics with respect to the starting materials and suitable for applications in this field.[10] Afterwards, different approaches were attempted to modify the mechanical characteristics of the materials. First of all, we changed the nature of the methacrylated polymers, so that hydrogels composed of gellan gum and polyethylene glycol dimethacrylate (GG-MA and PEG-DMA) were realized. The two polymeric systems were combined using the double network strategy (DN), a two-step procedure. The first step concerns the preparation of a stiff and brittle gel of a polyelectrolyte that is then immersed in an aqueous solution of a second monomer. After the diffusion, the monomer will be cross-linked, leading to the formation of a second, soft and ductile network inside the gel. This kind of system shows characteristic and mechanical properties where the high toughness is due to a synergistic effect of the binary asymmetric structure, rather than a linear combination of two component networks as it occurs in the conventional IPN.[11] These polymers were also combined together in order to create novel injectable hydrogels, easily delivered through a needle and photocross-linked in the injection site. Several concentrations and different molecular weights of PEG-DMA were investigated to modulate the composition of GG-MA hydrogels and overcome their extreme fragility.[12] As a valid solution to this problem, nanocomposite hydrogels (NC) obtained adding laponite were also proposed as wound dressing materials for the delivery of therapeutic agents. The improved mechanical properties of NC allowed its sterilization, usually impossible with conventional hydrogels and, as a consequence, its employment as innovative wound dressing material.[13] GG and the plasticizer glycerol (Gly) were then used for the preparation, by the casting method, of thin films suitable for the oral delivery of therapeutic molecules. The amount of Gly was varied in order to obtain films with tenable mechanical properties and high loading efficiency of fluconazole.[14] At low concentrations of Gly drug precipitation occurred while, for higher concentrations of Gly, a significant deterioration of mucoadhesive and mechanical properties was observed. To overcome all these problems hydroxypropyl-β-cyclodextrin (HP-β- CD) was added to the formulation as a drug- precipitation inhibitor. The effect of cyclodextrin addition on the properties of GG-Gly films was investigated. A sustained release was observed when a preformed complex fluconazole/HP-β-CD was loaded in the film.[15] Then, we decided to optimize and

characterize nanocomposite films based on GG-MAand silver nanoparticles (AgNPs) for application in the field of wound dressing. The films were produced using the solvent casting technique coupled with a photocuring process. The UV irradiation of GG-MA solutions containing glycerol as a plasticizer and different amounts of silver nitrate resulted in the concurrent crosslinking of the photocurable polymer and the reduction of Ag ions with consequent *in situ* generation of AgNPs. Microbiological tests confirmed the antimicrobial efficacy of the films, suggesting their possible application as dressings for the treatment of infected wounds. [16]

In 2012, the collaboration with dr. Petralito started as well as our research on liposomes. It is well known that stability issues are the main drawbacks in the pharmaceutical use of self-assembled lipid vesicles, so that we decided to increase the stability of these systems converting the internal structure of liposomes into a soft and elastic hydrogel. For the design of the gel-in-liposome systems (GiL), PEG-DMA was photopolymerized inside the core of hydrogenated soybean phosphatidylcholine/cholesterol liposomes. The inclusion of a PEG-DMA network within lipidic vesicles affected their intrinsic structural properties, improving the mechanical resistance of the assembled nanocarriers against chemical stress. The presence of the polymer did not hamper the possibility to remote load, using a transmembrane pH gradient, a weak acid model molecule (5(6)-carboxyfluorescein) as well as its release [17]. Then we decided to analyse the effect of the molecular weight of PEG-DMA on the main properties of the GiL systems. By varying the molecular weight of PEG-DMA, its hydrophilic/lipophilic balance was modified and different arrangements of the polymer within the structure of liposomes as well as different interactions with their membrane were obtained.[18] Release of (5(6)-carboxyfluorescein) was studied from conventional liposomes and GiL in a vertical Franz diffusion cell and a detailed transport model was proposed. The theoretical analysis of experimental release data strongly supports the basic assumption that, by varying the molecular weight of PEG-DMA, a different arrangement of the polymer within the liposomal structure and a different interaction with the membrane occur. [19] At the same time, our interest in the field of tissue engineering increased, directing towards other different polymeric networks. Cryogels are a particular type of hydrogels that possess great potential in both fields of drug delivery and tissue engineering as a spongy scaffold to promote the delivery of biomolecules. Precisely, the novel formulation was fabricated by combining dextranmethacrylate and PEG-DMA through radical polymerization at a temperature of -15 °C. The swelling, porosity, mechanical properties, and the drug release profile of vitamin B12 from the optimized cryogel were evaluated and compared to hydrogels fabricated at room temperature. The cryo-gelation technique produces the formation of scaffolds with improved interconnected porosity, and higher mechanical resistance than conventional hydrogels. [20] These structures showed a poor ability to promote cell growth so that a new polymer was considered. Gelatin methacrylate (GelMA) is a derivative of gelatin widely used in tissue engineering for its ability to promote cell growth. We proposed a new methacrylation procedure, carried out under homogeneous single-phase reaction conditions, which allows of obtaining a good reproducibility of the derivatization degree of the polymer in a simple and fast way, only on ε-amino groups of lysine and hydroxylysine. The effect of the reaction conditions on the rheological properties of GelMA was investigated and compared with those of the polymer obtained under traditional heterogeneous reaction conditions. The proposed single-phase synthetic protocol gave a polymer which behaves like a solution when used at 10% w/v concentration and even at temperatures aslow as 5 °C, whereas the polymer obtained with the classic biphasic procedure behaves like a gel within the same range of temperatures and when used at the same concentration.[21] These results are particularly interesting opening GelMA to new applications particularly in the biomedical field. Actually, after acquiring a 3D-bioprinter, all the polymeric derivatives we studied these 25 years, are under study in order to use them as inks (or better as base for bio-inks) in 3D- bioprinting.

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1973-2023 – 50th ADRITELF Anniversary

IV Convegno della divisione di Tecnologia Farmaceutica - SCI

Trieste September 11-13, 2023

DESIGN AND DEVELOPMENT OF DRUG DELIVERY SYSTEMS BASED ON SWELLABLE HYDROPHILIC POLYMERS

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Swellable hydrophilic polymers have extensively been leveraged in pharmaceutical formulation, particularly for the design and manufacturing of advanced therapeutic systems. In the area of oral delivery, hydrophilic matrices have represented one of the most popular strategies to achieve a prolonged release of bioactive compounds, which has long been pursued to reduce the dosing frequency and enhance patient compliance. In this respect, much effort has been made to achieve zero- order release kinetics that would be reflected in constant drug plasma levels throughout the treatment. Linear release profiles have been sought through a range of approaches, including swelling restriction, partial coating, special geometries and/or gradient compositions with inward increasing drug concentration [1]. In this respect, multi-layer hydroxypropyl methylcellulose (HPMC) matrices having non-uniform drug distribution, fabricated by powder-layering, were proposed to counteract the release slowdown due to progressive reduction of the surface area at the swelling front and lengthening of the distance drug molecules have to cover to diffuse outward [2,3]. As compared with a system having uniform composition, the gradient matrices were proved to enhance linearity of release through a simple design concept and an advantageous manufacturing technique, involving no solvents nor high-impact drying operations. In an attempt to aid the swollen polymer erosion, thereby limiting the increase in the gel layer thickness, the addition of cellulolytic enzymes to HPMC matrices was also explored [4]. This approach was shown to bring about synchronization of the swelling and erosion front movements over a relatively long time lapse and, unexpectedly, to mask the initial burst effect leading to early linearization of the release profiles.

Given the growing awareness of the key role played by timing in the success of a drug treatment, swellable hydrophilic polymers have been exploited to form functional barriers intended to defer the onset of release from a core unit for a programmable period of time. Notably, lag phases of proper duration preceding release would make chronopharmaceutical treatments possible for pathologies with night or early-morning symptoms, where intake of an immediate-release drug product would be associated with poor patient compliance [5]. Furthermore, lag phases exceeding the small intestinal transit time (SITT), which has been reported to be fairly consistent regardless of different dosage form types and fasted or fed state of the subjects, have been relied on for colon delivery purposes. To this end, enteric coating is required so that the influence of unpredictable gastric residence is circumvented, and time-based colonic release can ultimately be achieved [6]. Delivery of drugs, probiotics and prebiotics to the distal gut is of special interest for the therapy of inflammatory bowel disease (IBD), including ulcerative colitis and Crohn's disease, and of other pathologies of the large intestine, such as irritable bowel syndrome, infectious diarrhea, diverticulitis and dysbiosis. Moreover, mainly because of less abundant digestive proteases, the colon has been explored as site for non-invasive peptide administration [7-9].

The hydrophilic polymer barriers allowing for time-programmed release have been devised in the form of coating layers or of fillable capsule shells. The coatings have been applied to tablets, minitablets and gelatin capsules using different techniques: compression-coating, film-coating, both in top- and tangential-spray mode, and, more recently, powder-layering. Coated systems exhibiting satisfactory physico-technological characteristics and the desired release performance have finally been obtained. However, each of these techniques has involved pros and cons. Compression-coating yielded thick porous layers that were associated with lengthy and poorly fine-tunable delay phases [10]. Moreover, they proved unable to prevent a slow drug diffusion phase before the release pulse, particularly when high-viscosity HPMC grades were employed. This issue was faced by the use of cellulase, either added in admixture with the polymer or in a separate underlying layer [11]. As compared with a reference formulation devoid of enzyme, cellulase-containing delivery systems resulted in more reproducible profiles, with shortened lag phases and reduced diffusional release. Film-coating, due to the viscosity-building effect of the polymers, was challenging in setting up fully aqueous-based processes, so as to rule out the use of organic solvents, and in adapting operating conditions to a diverse range of substrates. With a selected low-viscosity HPMC grade (Methocel® E50), a successful outcome was reached in terms of process feasibility in a top-spray fluid bed, quality of the applied coatings, release-deferring ability of the coated tablets and modulation of the lag time depending on the coating level [12-15]. Nevertheless, the long process time required for application of the HPMC layer was an open issue.

From an investigation conducted to identify faster coating modes, tangential-spray aqueous film-coating and powder-layering were found toconsiderably reduce the process time and increase the relevant yield as compared with top-spray film-coating [16]. When the coated systems were obtained fromsmall-sized multiple-unit cores, an additional permeable film was needed to enhance the efficiency of the functional layer, compensating for the overall limited amount of HPMC applied [17]. In diabetic rats, insulin minitablets coated with HPMC, Eudragit® NE in admixture with superdisintegrant sodium starch glycolate, and Eudragit® L, respectively, led to a sharp rise in the plasma insulin concentration along with a marked decrease in the glucose level 6 h post dose, which was consistent with ileo-colonic position of the formulation [18]. The HPMC coatings were also combined with enteric films containing naturally occurring polysaccharides susceptible to selective microbial degradation in the colon, so as to provide single- and multiple-unit double-coated formulations having enhanced site targeting effectiveness as compared with delivery platforms based on exploitation of a single physiological variable of the gastrointestinal tract [19,20].

Fillable HPC capsules were fabricated as an alternative to HPMC-coated tablets by wet extrusion and hot-melt extrusion in early attempts, and by injection-molding as well as fused deposition modeling(FDM) 3D printing afterwards, assessing the potential of hot-processing techniques for pharmaceuticalmanufacturing [21-26]. These capsular shells were demonstrated to yield the desired pulsatile release performance and allowed for extemporaneous filling with a range of formulations, which would offer major regulatory benefits and also enable personalization of the therapy.

The *in vivo* performance of coated tableted systems was studied by pharmacokinetic, γ-scintigraphic and pharmacoscintigraphic techniques [13,27]. Concentration profiles with lag phases dependent on the coating level were obtained and, in the case of enteric coated formulations for colon delivery, disintegration was consistently seen in thelarge intestine. In a pharmacoscintigraphy study where 5-ASA-containing systems were administered, N- acetyl 5-ASA plasma levels were shown to rise in conjunction with colonic transit and disintegration of the dosage forms.

A different swellable hydrophilic polymer, polyvinyl alcohol (PVA), was exploited in view of the inherent water-induced shape-memory properties for prolonged drug release coupled with extended organ retention based on the size increase approach [28-30]. Prototype devices having different original and temporary shapes, enabling administration on the one hand and preventing fast emptying on the other, were fabricated by FDM, showing an interesting potential as stomach and urinary bladder indwelling delivery systems. For the same purpose, Organ-Retentive Osmotically-Driven Systems (ORODS) have recently been obtained by assembling drug-containing swellable matrices with osmotic units in the form of tubes made of semipermeable membranes closed at their ends. Due to the inflow of aqueous fluids, the osmotic compartments expand leading to an overall enlargement of the assembly. The organ-retentive devices proposed require no invasive removal procedures.

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IMPROVING ON NATURE: SUCCESSFULLY NANOCARRIERS FOR THE OPTIMISATION OF BIOPHARMACEUTICAL PROPERTIES OF NATURAL COMPOUNDS AND PLANT EXTRACTS

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There is an increasing demand for natural products and plant extracts, in particular for the new generation of science-based and standardized functional botanical ingredients to formulate herbal medicinal products and healthy products, mainly represented by medical devices, cosmetics and dietary supplements. This trend is principally due to numerous health benefits of natural products and plant extracts recently reported in the scientific literature, representing new therapeutic approaches or complementary and/or alternative treatments to the current medications, and a huge opportunity to meet consumer demand [1]. Numerous natural products are available on the market as pure constituents, together with conventional (using organic solvents) and innovative (supercritical CO_2 or subcritical water) extracts, in addition to increasing demand for essential oils. Extracts and essential oils are generally very complex mixtures made of molecules with different solubility and chemical structures. In truth, numerous isolated constituents and extracts, as well as essential oils, need repeated administrations or high doses to be used in clinical practice because of low hydrophilicity and intrinsic dissolution rate(s) or physical/ chemical instability. Other drawbacks are low absorption, poor pharmacokinetics and bioavailability, scarce biodistribution, first-pass metabolism, trivial penetration and accumulation in the organs of the body. Consequently, their impressive *in-vitro* potential demonstrates less or negligible *in-vivo* activity due to their poor solubility or improper molecular size, resulting in poor absorption and hence poor bioavailability [2-4].

Currently, drug delivery systems represent a useful tool for enhancing the bioavailability of isolated natural products, extracts and essential oils. The novel formulations have remarkable advantages over conventional formulations of plant actives and extracts, which include increased solubility, bioavailability, enhancement of pharmacological activity and intracellular uptake, modification of pharmacokinetics and biodistribution, improved tissue macrophages distribution, sustained delivery and protection from physical and chemical degradation [2,4].

In our studies, polymeric nanoparticles, lipid-based nanocarriers including micelles, vesicles, nanocochleates, micro- and nanoemulsions, self microemulsifying drug delivery systems, cyclodextrin complexes, in addition nanocarriers loaded in hydrogels, and solid dispersions and co-ground products were investigated to overcome biopharmaceutical shortcomings of isolated active ingredients, extracts and essential oils.

The investigated active ingredients were isolated natural products (andrographolide, berberine, cannabidiol, curcumin, resveratrol, silybin, artemisinin, thymoquinone, khellin), extracts (*Vitex agnus-castus*, *Sylibum marianum*, *Hypericum perforatum*, *Olea europea*, *Serenoa repens*) and essential oils (from *Origanum*, *Salvia*, *Melissa*, and *Artemisia* species). This presentation reports on some selected success stories of drug delivery systems developed in our research group and based on natural products, extracts and essential oils. Remarkable advantages over conventional formulations, namely, increase of solubility, stability, permeation and bioavailability, and sustained delivery, were found [5-12].

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PROGRESS OF PHARMACEUTICAL TECHNOLOGICAL AND COSMETIC RESEARCH AT GENOA UNIVERSITY

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The progress in pharmaceutical technology at Genoa University has evolved in parallel with the advancement and consolidation of ADRITELF association, of which Prof. Gaetano Bignardi was one of the founding members and a staunch supporter. At the beginning, technological research took a slow start while remaining closely linked to the chemical-pharmaceutical approach. Noteworthy are the latency studies with the use of prodrugs of [1,5]-benzodiazepines [1], the development of cis-platinum and procaine complexes for the treatment of ovarian tumors [2] and the study of surfactant activity of some natural products [3]. Between the 80s and 90s, thanks to a slow and constant acquisition of resources, Genoese technological research branched out to different fields, mainly concerning mucoadhesion, solid state, nanoparticles/nanotechnology and radiopharmacy.

Mucoadhesive dosage forms have been developed both for topic and systemic administration. Discs for transmucosal oxycodone delivery based on gelatin have been developed and by a clinical study on cancer patients and a single dose pharmacokinetic study in healthy volunteers proved their therapeutic potential[4]. A buccoadhesive film based on low methylated pectin for sublingual administration of oxycodone for acute pain control was also prepared and fully characterized [5]. In another study, a mucoadhesive microsphere formulation, based on cross-linked amylose starch with sodium trimetaphosphate, was optimized for the peroral administration of 5- fluorouracil [6]. Buccoadhesive systems in the form of polymeric films and reservoir systems based on HPC and tablets prepared with mixtures of chitosan and poloxamer have also been proposed [7]. Mucoadhesive vaginal gels based on HEC were prepared and characterized for an industrial partner [8]. In mucoadhesion studies the problem of the reference substrate used in the adhesiveness test has been addressed, because animal mucosa has high cost and high variability while artificial substrates often give unreliable results, therefore the use of egg-shell membrane was proposed as a biological standard substrate [9].

In the field of mucoadhesive tablets, a number of matrixes have been analysed, including one based on chitosan lactate and poloxamer, incorporating Eudragit microparticles, to increase the residence time of antimicrobial and antimycotic agents, submitted to a clinical trial [10], and another containing different types of chitosan salts in association with poloxamer P407 [11]. A mucoadhesive system has been obtained from the application of a thermal treatment to direct-compression tablets including polyacrylate polymers, which, as a consequence of heating, form a monolithic, hydrophilic, swellable matrix able to control the drug release. This patented technology [12-13] has been exploited to obtain a gastroretentive formulation, able to float on the gastric content, controlling the release of various actives. The promising results obtained in vitro with this licensed technology will have to be evaluated through a clinical trial [14]. One analogous technology has been adopted to develop a polyacrylic hydrogel matrix suitable for 3D cell culture. These patented scaffolds have been successfully employed for the culture of various cell lines, for time spans longer than conventional 2D cell cultures [15].

In the field of solid state, studies on polymorphism [16] preformulation, inclusion complexes [17] and stability [18-19] have been carried out, always paying particular attention to the design of the experiments and the validation of the results through a statistical approach and to result optimization by DOE methodologies [20-21].

Since the early 2000s, nanoparticulate therapeutic systems have also been object of research activity. Nanoparticles were prepared through electrostatic interaction of an anionic cisplatin-alginate complex with chitosan or N-trimethyl chitosan and resulted able to exert cytotoxic activity in vitro on murine and human cancer cell lines [22]; on the basis of these promising results, alginate was replaced with hyaluronate, yielding nanoparticles that showed good anticancer efficacy in mice and reduced toxicity [23]. Nanoparticles with antiviral activity were obtained by ionotropic gelation of chitosan induced by foscarnet, acting both as the active substance and as an ionotropic agent, and were crosslinked with glutaraldehyde. Foscarnet released from these nanoparticles maintained the antiviral activity of the free drug when tested in vitro against infected lung fibroblasts, potentially improving its therapeutic effect [24]. An intra-articular delivery system constituted by a poloxamer gel vehiculating clodronate in chitosan nanoparticles was conceived to obtain a specific and controlled release of clodronate in the joints to reduce the arthritis rheumatoid degenerative effect [25]. Another injectable formulation based on two different poloxamers, one of which undergoing a sol-gel transition at body temperature, has also been proposed for the sustained release of metformin hydrochloride, a drug recently repurposed for antitumor activity: the formulation exerted a significant reduction in tumor growth and neoangiogenesis in mice pseudo-orthotopically grafted with human breast cancer cells [26].

Sterically stabilized liposomes including fenretinide, a synthetic retinoid featuring anticancer activity, and decorated with NGR peptides for targeting the tumor endothelial cell marker, aminopeptidase N, were successfully tested in mice [27], inspiring the development of fenretinide-loaded mesenchymal stem cells-derived extracellular vesicles to be applied in neuroblastoma. This research line has been extended with all-trans-retinoic acid-loaded micelles made of D-α-tocopheryl polyethylene glycol succinate; the micelles, loaded into carbopol gel, confirmed their cytotoxic effect on melanoma cells [28]. Finally, a bortezomib-loaded liposomal formulation, including an amino-lactose as complexing agent to entrap the drug inside the internal aqueous compartment, was set-up and characterized; the vesicles showed anticancer activity, while minimizing drug side effects, in orthotopic neuroblastoma-bearing mice [29].

Another research line is focused on the development of new agents for radioactive imaging in vivo. In this context, an agent potentially suitable for application in the Radio-guided Occult Lesion Localization of non-palpable mammalian cancerous lesions has been obtained by conjugating human albumin with the macrocyclic chelator DOTA, resulting in a microsized water-insoluble powder aggregate, stable under the thermal conditions adopted for radiolabelling; a test on mice after labelling with ^{64}Cu proved its capacity to keep the radioactive isotope located in the inoculation site for at least 40 h [30]. Also, two novel Positron Emission Tomography radiopharmaceuticals have beendeveloped for imaging of early atherosclerotic lesions, both being based on a peptide binding VCAM-1, a protein overexpressed on early atheromas: one derived from the direct conjugation of a DOTA derivative with the peptide, the latter was a biotin derivative conceived to be employed in a 3-step biotin-avidin pretargeting system. The two systems evidenced their VCAM-1 binding ability in vitro on human umbilical vein endothelial cells [31]. Currently, a new researchactivity has been addressed to the development of loaded biomimetic vesicles and liposomes, also to maximize the accumulation of ^{10}B isotope in cancer cells for boron neutron capture therapy [32].

At Pharmacy department the teaching of cosmetic science and the related research is being conductedsince the end of the 70s [33]. In the latest twenty years the research has focused on Green Cosmetic Chemistry, with particular attention to microwave technology applied to eco-friendly procedures [34]. The main achievements have been obtained in solvent- free organic reactions for the synthesis of cosmetic ingredients [35], microwave-mediated hydrothermal processes for the preparation of inorganic [36] and organic-inorganic hybrid materials [37] and solvent-free microwave extraction of bioactive compounds from botanical matrices [38] and agrifood waste [39]. In 2015, the results of this topic led to a technology transfer [40] with the constitution of an innovative start up, University Spin off ACCADERMICA®, whose project was presented at the prestigious EXPO 2015.

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Tuesday, September 12

Chairpersons *Patrizia Santi*

14.30-15.10 Prof. Sophia Antimisiaris University of Patras
Targeted liposomes for drug delivery applications, systemic and local administration

PLENARY LECTURE

Targeted liposomes for drug delivery applications, systemic and local administration

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The application of nanotechnology in the development of sophisticated formulations for drug administration and/or targeted delivery, has led to the development of liposomal nanotherapeutics with exceptional advantages. Despite the important recent milestones that facilitated clinical approval of the first liposomal nucleotide drug and development of liposomal mRNA vaccines, several challenges are still present.

One of the current challenges is the potential to target specific diseases and/or overcome biological barriers with ligand-targeted liposomes (a technology that has not been yet translated into pharmaceutical products).

Another question being investigated in out lab is if liposomal drugs may present advantages to overcome the serious problem of antimicrobial resistance.

Herein the approaches/findings of the last years in our lab to overcome current challenges and/or investigate potential solutions for unmet medical needs using liposomal drugs will be presented.

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Prof. Sophia G. Antimisiaris is a full Professor and Director in the Pharmaceutical Technology Lab, Dept. of Pharmacy, University of Patras, Greece and a collaborating member of the Institute of Chemical Engineering of the Foundation of Research and Technology, Hellas (FORTH/ICE-HT). She received her M.Pharm and PhD degrees from Department of Pharmacy, University of Athens, GR (1984 and 1988). After postdoctoral training at the School of Pharmacy, USC, Los Angeles, CA, she joined University of Patras. In 1992-1993 she was a Visiting Professor in the Centre for Drug Delivery Research (CDDR) in the School of Pharmacy, University of London, UK, where she worked with Prof. Gregory Gregoriadis. She currently serves as an Associate Editor of *J. Liposome Research* and editorial board member of the Journals *Pharmaceutics*, *Drug Delivery*, and others. She is currently the coordinator of the H2020 project Smart Bone Regeneration (SBR), and a PI in the EURONANOMED 2021 project NANO4STROKE.

Tuesday, September 12
Session 5

Room A - Sala Tergeste

Chairpersons *Stefano Giovagnoli – Elisabetta Esposito*

15.10-15.35 Technological platforms for ocular drug delivery

Rosario Pignatello, University of Catania

15.35-16.00 Enhancing drug transport across biological barriers: polymeric micelles for skin, buccal and ocular drug delivery

Sara Nicoli, University of Parma

16.00-16.25 Past innovations and future perspectives in ophthalmic drug delivery-an update on nanosystems

Patrizia Chetoni, University of Pisa

16.25-16.55 Coffee break

1973-2023 – 50th ADRITELF Anniversary

IV Convegno della divisione di Tecnologia Farmaceutica - SCI

Trieste September 11-13, 2023

TECHNOLOGICAL PLATFORMS FOR OCULAR DRUG DELIVERY

R. Pignatello, C. Puglia, M.G. Sarpietro, T. Musumeci, C. Carbone, L. Montenegro, A. Bonaccorso, D.C. Santonocito

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Although ophthalmology can be considered as a 'niche' segment of the pharma field, acute and chronic pathologies of the eye are perceived as a strong prejudice and threatening of the quality of life of the involved patients. According to the latest GBI Research report "Global ophthalmology drugs market to 2023", the ophthalmology market has grown up to present exceeding USD 35 billion a year.
The most common pathologies concern glaucoma, age-related macular degeneration, macular oedema, retinopathy and dry eye, plus allergic conjunctivitis and retinitis pigmentosa. The largest number of developing drugs is concentrated around the first two.
The research data show that ophthalmology is the 9th most important therapeutic area in the world with about 850 products under development and six of the eight most important ophthalmic companies are also on the list of the top 20 worldwide in the pharma field. Despite that, only 5% of the pipeline of these big pharma concerns eye diseases. Therefore, many analysts predict a strong rise of small specialized companies.

A major challenge in ocular drug therapy is to improve the poor bioavailability of topically applied ophthalmic drugs by overcoming the severe constraints imposed by the eye on drug absorption. Therapies of common eye diseases are usually associated with well-known technological limits:
- topical instillations used for the anterior segment display short drug residence time, drainage and need of frequent applications;
- therapies addressed to the posterior segment usually require invasive means or must face the low-permeability of periocular routes.
Alternative strategies for eye diseases treatment are thereby continuously proposed to address such limitations. Among them, the large experience gathered in drug delivery technologies and nanomedicine can be successfully applied to this clinical field.

Colloidal (nano-sized) ocular DDS (ODDS) can ensure important outcomes in ocular drug delivery, both from a technological point of view (e.g., increased topical drug bioavailability, controlled/prolonged drug release, etc.), for applicative reasons (e.g., reduced frequency of instillation, reduced irritation, etc.), and from a clinical landscape (improved uptake by corneal cells, reaching/targeting to specific areas/tissues, such as retina, reduction of the required dosage and of systemic side-effects, etc.) [1,2].

However, due to their peculiar structure and functions, eye tissues require an *ad hoc* optimization of the formulations to be applied on the eye surface or injected/inserted in the posterior segment. A deep evaluation of scaling up likelihood must be made at a very early stage of development.

The NANO-*i* Research Center at the University of Catania (now conveyed into the NANOMED Research Center) has been settled in these last years to perform basic and industry-oriented researches in the field of controlled/targeted ocular delivery of drugs and bioactive compounds.
Through spontaneous studies as well as company-required researches, a wide range of (nano) technological platforms have been exploited with some success, with the specific aims of delivering active compounds either on the eye surface or to the posterior segment.
Among the first examples, nanomicelles, nano-emulsions, SNEDDS and polymeric nanoparticles have been largely investigated for the delivery of drugs such as melatonin, antibacterials, NSAIDs, along with some natural compounds. To reach the retinal area, conversely, systems based on lipid materials, and in particular nanostructured lipid carriers (NLC) and drug-lipid nanoconstructs (LDC) have shown the capacity of ensuring a rapid and prolonged presence of drugs in the vitreous and in the retina, most probably through a trans-scleral diffusion of the nanocarrier from the ocular surface to the back of the eyeball.
A crucial aspect of these studies, and in particular of those linked to the requirements of industrial partners, has been the selection of highly safe ingredients (i.e., polymers, lipids and surfactants with a GRAS status or approved by EMA), the use of safer ICH Class-3 solvents, the development of even more 'green' production methods that could be easily importable and scalable at an industrial level.

Therefore, a mere translation of production methods from other therapeutic fields into the ophthalmic therapy cannot be foreseen, for instance due to technological constrains such as the requirement of sterility and isotonicity, just to cite some of them. A useful strategy that our group has established is the optimization of formulation composition and preparation using a Design of Experiment (DoE) approach, by means of the Design-Expert® software [3].

Regulatory issues in the field of nanomedicine are also a critical issue in the industrial application of laboratory outcomes.
Last, but often not least, the 'fashion-driven' desiderata of pharmaceutical companies, that ask to develop 'original' nanotech ocular formulations, often condition the technological strategy to be adopted.

Examples of recent projects developed by our research group are cited among the References [5-12].

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A dutiful acknowledgement must be given to the colleagues, now retired, who greatly contributed to this research team: prof. Giovanni Puglisi, Francesco P.Bonina and Francesco Castelli.

ENHANCING DRUG TRANSPORT ACROSS BIOLOGICAL BARRIERS: POLYMERIC MICELLES FOR SKIN, BUCCAL AND OCULAR DRUG DELIVERY

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Despite the ease of application and the significant advantages related to low systemic side effects, local drug delivery still represents a challenge. This is mainly due to the tough barriers that the drug needs to overcome to reach the target site. These barriers, which differ in thickness, histology and composition depending on the tissue considered, often prevent the drug to reach clinically relevant concentrations at the target site. The research carried on by our Group has been dedicated to the setup of reliable *in vitro* and *ex vivo* models considered as powerful tool in the development process of topical formulations [1, 2]. Particularly, the focus was on the comprehension of the mechanisms governing drug transport and on the use of enhancing strategies to foster the drug transport across biological barriers, namely skin, buccal mucosa and ocular tissues [3-5]. Special attention has been dedicated in the last 10 years to high molecular weight compounds, such as proteins and oligonucleotides [6-8], investigating the possibility to achieve significant tissue accumulation and permeation, thanks to the use of chemical enhancers [8], iontophoresis [2, 9] and cell penetrating peptides [10]. The knowledge acquired on the barrier properties of biological membranes together with the elucidation of mechanisms of drug penetration and enhancement has represented the basis for the development of innovative formulations, including films, microemulsions and polymeric micelles [11-17].

Polymeric micelles represent interesting carriers for local drug delivery. Despite their potentiality, several aspects deserve further clarification, mainly with respect to their stability, mechanism of action and interaction with biological barriers. We have evaluated their potential for drug administration to ocular tissues, by using primarily TPGS (tocopheryl polyethylene glycol 1000 succinate) as micelle-forming polymer. The studies have demonstrated the capability of these micelles to enhance ocular absorption of several lipophilic drugs used in the treatment of diseases affecting both the anterior and the posterior segment of the eye, such as cyclosporine, simvastatin, econazole and dexamethasone [15, 18]. Micelles have demonstrated a very good performance on conjunctiva, cornea and scleral tissue. Important aims were also the identification of their penetration mechanism into cornea and sclera as well as of their ability to tune drug release once inside the tissues. At this regard, the association of several techniques, such as the quantification of drug and polymer in tissues, the evaluation of TPGS hydrolysis in the presence of esterase and two-photon microscopy, allowed us to highlight different behaviors depending on the nature of the tissues involved (Figure 1).

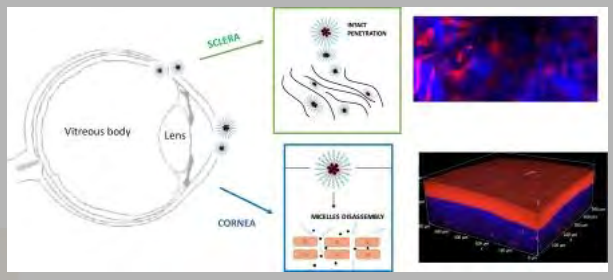

Figure 1. Fate of TPGS micelles in contact with sclera and cornea. From ref [15] with permission.

A similar approach was also used to investigate the potential of this nanocarrier for the skin delivery imiquimod, an immunostimulant compound agonist of the toll-like receptors 7 and 8 used in the treatment of precancerous and cancerous skin lesions. In this case, we exploited the co-loading of hydrophobic compounds, such as fatty acids and their esters, to increase the solubilization ability of TPGS micelles toward imiquimod. Oleic acid co-loaded micelles were stable and favored drug penetration across the stratum corneum and accumulation in hair follicles. Indeed, loading of fluorescent probe in the micelles permitted to visualize the penetration pathway and to identify micelles disruption as the main drug release mechanism in the skin tissue [14].

Despite the undeniable potential, the poor viscosity of micellar formulation would reflect in a limited retention time at the application site. Therefore, the research was also dedicated to figure out effective approaches to control the persistence of micelles on the administration site by including them either in gels or in polymeric films. The preparation of micelles-loaded polymeric films took advantage from the long lasting expertise of our group on the development of hydrophilic patches for dermal and transdermal delivery [19]. We found that the capability of TPGS micelles to diffuse across gels depended upon gel composition more than upon gel macroviscosity. Micelles-loaded gels and polymeric films were evaluated for the buccal delivery of imiquimod, for the treatment of oral cancers [11]. Films made of xanthan gum and alginates showed good

mucoadhesion properties, controlled the release of the drug and enhanced its retention in the mucosa, minimizing at the same time the transmucosal permeation, which could be responsible for systemic absorption and side effects (Figure 2).

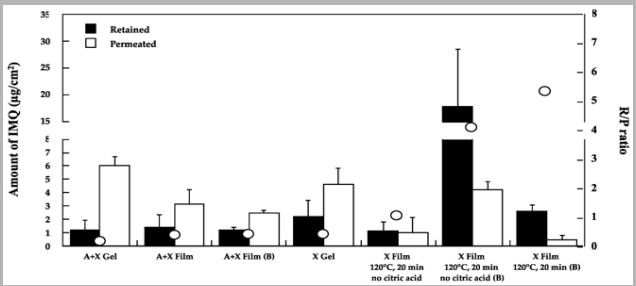

Figure 2. Amount of imiquimod accumulated in the mucosa and permeated, starting from TPGS micelles loaded in different gel and films. From ref [11]

Presently, we are investigating the possibility of tuning micelles release from hydrophilic polymeric films made of xanthan gum and polyvinyl alcohol by modulating the crosslinking. The harsher crosslinking conditions, associated to a lower swelling degree, were unexpectedly associated with a faster release of TPGS micelles for comparison with milder conditions. The inclusion in the film of drugs with low affinity for the micelles resulted in an immediate drug release, independent by crosslinking conditions and film swelling, while the loading of molecules with high affinity for the micelles' core resulted in a controlled release, tunable by the modulation of the crosslinking. Finally, the ongoing research on polymeric micelles is also devoted to understand the role of PEG chains length, constituting the corona, on micelles characteristics, solubilizing properties and diffusion behavior across biological barriers.

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PAST INNOVATIONS AND FUTURE PERSPECTIVES IN OPHTHALMIC DRUG DELIVERY-AN UPDATE ON NANOSYSTEMS

P. Chetoni, S. Burgalassi, D. Monti, S. Tampucci

Università di Pisa, Dipartimento di Farmacia

As in the past, ocular drug delivery is still a challenge for pharmaceutical technologists and particular attention is paid to the development of innovative formulative approaches, including nano- systems.

The presence in the eye of various static and dynamic physiological barriers capable of protecting the ocular structures from the entry of xenobiotics, prevents both the maintenance of the ocular formulation at the site of absorption and the active absorption of therapeutic agents in the internal structure of the eyes.

The design of an ideal ophthalmic delivery system should include the enhancement of drug bioavailability and the controlled release of drug at the site of action, overcoming the various ocular barriers. In addition, the rational design of an ophthalmic delivery system requires the maintenance of therapeutic drug levels for a longer duration in target tissues.

Topical instillation is the widely preferred non-invasive route of drug administration, especially for the treatment of diseases affecting the anterior segment. Conventional dosage forms such as eye drops have historically accounted for 90% of marketed ophthalmic formulations as they promote patient compliance, while intravitreal injections represent the main treatment for posterior segment disorders [1].

Most of the efforts in the field of drug delivery have been addressed to the study of viscosity enhancers to improve the precorneal residence time of the formulation and the choice has often fallen on the selection of mucoadhesive polymers. The use of a natural and semi-synthetic water-soluble polymers, including *in situ* gelling polymers in viscous semi-solid preparations, to enhance the contact time and also the drug penetration for the interaction with mucus layer or ocular tissues has been a successful strategy [2].

A parallel development strategy including the increase of the contact time, the viscosity/mucoadhesive-enhancement and the control of the drug release rate has been achieved with ocular inserts design, which remains a promising approach for ocular drug delivery targeting the back of the eye (vitreous humor) [3].

Recent developments in nanotechnology may provide an advantageous solution to overcome the drawbacks and limitations of traditional drug-delivery systems, above all low drug permeation through ocular barriers.

Nanosystems for ocular drug delivery, consisting of nanoparticles, liposomes, solid lipid nanoparticles (SLN) and nanomicelles, generally show high patient compliance.

Nowadays, there appears a huge interest in the nanomicellar strategy probably due to their high drug encapsulation capacity, ease of preparation, small size, and presence of a hydrophilic corona resulting in an improvement of the aqueous solubility of the lipophilic drug [4].

Assembling Surfactants-Mucoadhesive Polymer Nanomicelles (ASMP-Nano), based on a binary system of two surfactants (Kolliphor®TPGS and Igepal®CA- 630 in combination with hyaluronic acid, chosen as mucoadhesive polymer, has been developed and represents a successful research project.

The Cyclosporin-A nanomicellar system showed a prolonged drug retention time in the precorneal area of the eyes, protective effect towards epithelial corneal cells with a cell viability of more than 80% and a strong interaction with cellular barriers into the cells as evidenced by fluorescent probe distribution.

The same type of nanomicellar formulation was incorporated into the polymeric dispersion to prepare hybrid inserts by the solvent casting method. After characterization and evaluation in term of in vitro release of CyA, eye irritation potential, nanomicelles distribution inside the insert and in vivo pharmacokinetic, the advantage of this approach has been confirmed [5].

Another formulation based on Kolliphor®TPGS and Kolliphor®RH-40 was incorporated into optimized ion- sensitive polymeric dispersions of gellan gum (GG-LA) able to trigger the sol–gel transition after instillation. This new combined approach allowed the development of a clear aqueous dispersion, able to form a viscous gel in contact with the tear fluid, improving the ocular bioavailability of CyA.

In the future further studies could widen the treatment options for other ocular districts including the back of the eye.

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Tuesday, September 12
Session 6

Room B - Sala Zodiaco

Chairpersons *Piera di Martino – Gaia Colombo*

15.10-15.35 Pharmaceutical technology 2.0: innovative perspectives for the formulation of drugs at the molecular-level

Dritan Hasa, University of Trieste

15.35-16.00 New insights into solid lipid microparticles for oral administration produced by spray congealing technology

Nadia Passerini, University of Bologna

16.00-16.25 From pharmaceutical technique to pharmaceutical technology in 50 years at the university of Padua

Nicola Realdon, University of Padova

16.25-16.55 Coffee break

PHARMACEUTICAL TECHNOLOGY 2.0: INNOVATIVE PERSPECTIVES FOR THE FORMULATION OF DRUGS AT THE MOLECULAR-LEVEL

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Europe is facing four main healthcare challenges: (i) the rising and potentially unsustainable health and care costs, mainly due to the increasing prevalence of chronic diseases and to an ageing population requiring more diversified care (ii) the influence on health of external environmental factors including climate change; (iii) the risk to lose our ability to protect the populations against the threats of infectious diseases; (iv) health inequalities and access to health and care. There is, therefore, an urgent need for innovative medicines and pharmaceutical technologies that can satisfy as many as possible of the aforementioned challenges. In this context, an increasing number of medical applications and products containing nanomaterials, or at least with nano-based claims, have become available. This also happens in different areas of pharmaceuticals. In fact, the use of nanotechnology in the development of new medicines is now a main argument for different important research laboratories in the European Union (EU) and it has been recognized as a Key Enabling Technology, capable of providing new and innovative medical solution to address unmet medical needs. [1]

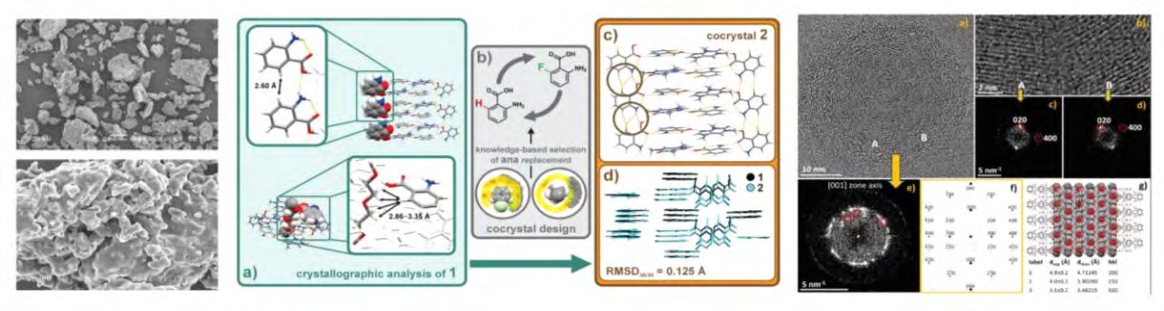

Figure 1. From particles (left) to single molecules (centre): a top-down view of a solid using advanced characterization techniques such as high-resolution (right) TEM. Adapted from ref [5].

Pharmaceutical nanotechnology represents nowadays one of the most interesting approaches for solving various issues related to drug delivery, including the improvement of the solubility of hydrophobic drugs, stabilization of degradable compounds, and the targeted and localized delivery. The introduction of crystal engineering strategies for developing new medicines represents another important topic. In general, there are several supramolecular synthetic strategies for the design and construction of complex supramolecular entities. Such strategies can be divided in two main categories namely (i) synthetic strategies based on a stepwise build-up of the multicomponent solid starting from precursors with heteromolecular recognition and (ii) synthetic strategies that uses the "cocktail" approach for obtaining the desired stoichiometric multicomponent solid. The strategies have already been successfully used for the design of new multicomponent solids [2]. Additionally, the possibility to produce such innovative drug delivery systems through environmentally-friendly processes will further increase the relevance of this field. Indeed, mechanochemistry, the use of mechanical force to induce and sustain chemical transformations has recently been highlighted by IUPAC as "one of the ten most important chemistry innovations that will change the world". Although such discipline is relatively new with a significant growth of interest observed particularly over the last three decades, several independent studies have demonstrated mechanochemistry to be effective and often superior to other approaches for the discovery of new solid forms [3]. For example, the propensity of a specific molecule to give different polymorphs and/or form multicomponent crystals can be assessed mechanochemically by changes in the exact conditions of the reaction [4].

This presentation has particular focus on the most recent developments of innovative medicines obtained through mechanochemistry, and designed using crystal engineering strategies, and relates the outcomes both to the experimental conditions and to the chemical characteristics of the compounds. Some relevant examples already available in literature will be mentioned, and the most recent results in our laboratory will be also presented.

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NEW INSIGHTS INTO SOLID LIPID MICROPARTICLES FOR ORAL ADMINISTRATION PRODUCED BY SPRAY CONGEALING TECHNOLOGY

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PharmTech Lab, Department of Pharmacy and BioTechnology, Alma Mater Studiorum- Università di Bologna

Solid lipid microparticles (SLMs) are micro-scaled drug delivery systems possessing a matrix made of high melting points lipids, such as fatty acids, glycerides, fatty alcohols, or solid waxes. Lipid excipients present important advantages, such as versatility, low cost and biocompatibility; they are completely tolerated by the body due to their non-cytotoxic and biodegradable nature.

Spray congealing, also known as spray chilling or spray cooling, is a process that transforms a lipid melt into well-defined spherical SLMs (particle size in the range 50–500 µm). During the atomization step, the molten fluid stream breaks up into small droplets; the spray solidifies upon cooling forming solid particles. The main advantage of this technology is the absence of solvent, either aqueous or organic, with related benefits such as the possibility to load hygroscopic and water-sensitive substances and no toxicity related to the presence of organic solvents. Other advantages include the ability to obtain spherical free-flowing particles suitable for tableting or capsule filling without the need of other downstream processes (e.g., secondary drying, milling) and high encapsulation efficiency values [1]. This technology has been used by our research group since the end of the 90s. Over the years, different types of small molecules have been encapsulated into SLMs aiming to modify their release after oral administration. According to the nature of the selected excipients, SLMs produced by spray congealing have been employed either to extend the release of drugs, to mask the unpleasant taste of some substances, or to protect labile drugs against degradation inside the gastrointestinal (GI) tract.

In the last five years, we have focused our attention on SLMs as innovative vehicle for the **oral administration of biological molecules**, such as enzymes (β-galactosidase and catalase) or peptides (glutathione) in order to obtain a prolonged release and/or gastric protection of the biological molecules. Oral local delivery of therapeutic biologics is generally limited due to the multiple obstacles of the GI tract, mainly represented by acidic stomach pH and digestive enzymes. In 2018, spray congealing was used to prepare SLMs loaded with β-galactosidase (lactase) in order to obtain a lipid-based solid oral formulation able to protect the acid-labile protein from gastric pH and from digestive peptidases and alongside to deliver it to its physiological site of action, the small intestine [2]. Among lipid-based excipients, we evaluated some commonly used long-chain glycerides and their digestion in presence of physiological lipases was investigated. Due to a fine balance between lipophilicity (low HBL) and digestibility, glyceryl trimyristate showed the best performance as carrier for SLMs. Lactase-loaded SLMs showed with very good encapsulation efficiency (>95%) and the formulation was able to protect the enzyme against gastric pH and pepsin up to 70%, as shown in Figure 1. Meanwhile, it could be effectively emulsified by the bile salts and then digested by the intestinal physiological lipases, in order to selectively release the macromolecule directly in the site of action (Figure 1) [2].

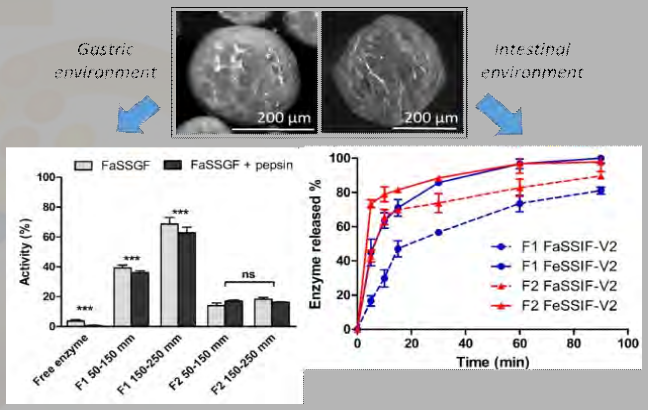

Figure 1. SEM images of lactase-loaded SLMs and their behaviour in simulated gastric and intestinal environments. From [2].

A more in-depth investigation about the structural integrity of a model protein, catalase, after its encapsulation in glyceryl trimyristate-based SLMs was investigated in a later study by means of circular dichroism and fluorescence spectroscopies [3]. The results confirmed that no conformational change occurred during the production process and both the secondary and tertiary structures were retained. Although highly sensitive to temperature, catalase retained most of its biological activity after encapsulation in SLMs, due to the loading of the drug at the solid state that reduced the risk of protein denaturation. Catalase activity after exposure to simulated gastric conditions (considering both acidic pH and presence of digestive hydrolases) ranged from 35 to 95% depending on the carrier: the increase of both the fatty acid chain length and the degree of substitution of the glyceride enhanced residual enzyme activity [3]. Moreover, SLMs containing glutathione, alone or together with catalase, were effective in reducing intracellular reactive oxygen species levels[4]. Overall, these studies indicated that spray

congealed SLMs are a promising oral delivery system for biotherapeutic products.

Despite the advantages of SLMs, the **issue of polymorphism** is a common problem of lipid-based delivery systems produced by melting technology and represents still a challenge in the pharmaceutical industry. Thus, during the last two years we have been investigating the impact of different liquid lipids (LL) on the polymorphism, structure and release behaviour of SLMs, using tristearin as model lipid. During the production process, tristearin SLMs crystallized in the unstable α-form and were subjected to a slow phase transition to the stable β-polymorph during storage. Small amounts (5 % w/w) of LL with different chain length were found to significantly speed up the polymorphic transition of tristearin from α to β-form [5]. The kinetic of phase transition induced by LL on SLMs was successfully monitored by synchrotron SAXS/WAXS analysis at Elettra Sincrotrone (Trieste) while simulating the melt-cooling process (Figure 2). The results evidenced the effect of additives ininducing the growing of a second lamellar β-phase compared to pure tristearin, already after few minutes from solidification. Moreover, synchrotron data gave important structural information about the effect of LL on the lipid lamellar thickness and crystallite size. Overall, the addition of LL can be considered an interesting approach to enable the production of SLMs in the stable polymorphic form. From the industrial viewpoint, this approach might be advantageous as any polymorphic change will be complete before storage, hence addressing the issues of drug release modification due to solid state transitions during storage.

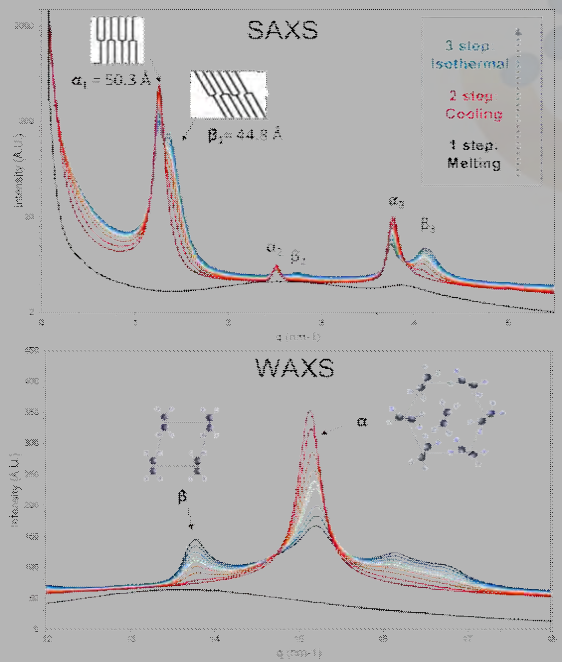

Figure 2. Synchrotron SAXS/WAXS analysis of tristearin SLMs with LL during melt-cooling process (unpublished data).

In parallel, we have explored for the first time the possibility to use the spray congealing technology for the production of lipid-based systems with more complex structures. Hence, instead of a lipid matrix, hybrid or multicompartment particles composed of lipids coupled with a different type of material (e.g. polymers such as PEG or Poloxamer) have been produced, transforming a melt emulsion into solid particles with a **biphasic structure (b-MPs)**. The external and internal morphology of b-MPs was successfully characterized by a combination of confocal microscopy (CLSM), SEM and Raman imaging, revealing solid particles with different architectures (Figure 3). In particular, microemulsions led to the formation of particles with a homogeneous structure, while coarse emulsions generated *multicores- shell* particles consisting of hydrophilic cores distributed within a crystalline lipid phase. Depending on their composition and structure, b-MPs could achieve various release profiles, representing a more versatile and tunable system than SLMs based on a single lipid phase [6].

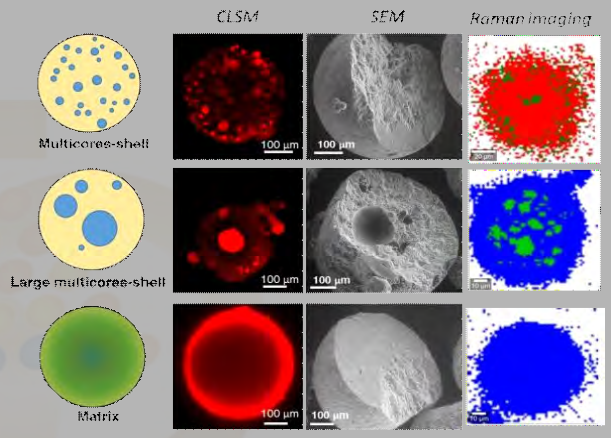

Figure 3. Spray congealed biphasic MPs with different structure characterized by various techniques (CLSM, SEM of cross-sectioned MPs and Raman imaging). From [6].

Concluding, we have illustrated the most recent researches of our group concerning SLMs for oral administration produced by spray congealing. We have studied new applications and unexplored aspects of this technology including: encapsulation and protection of biomolecules from various degradation sources; addition of additives to address the issue of solid state transitions during storage; the potential of producing microparticles with a biphasic structure.

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FROM PHARMACEUTICAL TECHNIQUE TO PHARMACEUTICAL TECHNOLOGY IN 50 YEARS AT THE UNIVERSITY OF PADUA

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Università degli Studi di Padova, Dipartimento di Scienze del Farmaco

50 years of ADRITELF almost coincide with the consolidation of research in the pharmaceutical technology field started by Prof. E. Ragazzi, since he has been entrusted by Prof. L. Musajo at the beginning of 1970s with the development of the pharmaceutical technique which evolved into the current meaning of pharmaceutical technology.

The wide diversification of the studies with which the research topics have been consolidated requires us to mention only a few of them.

In the context of *pharmaceutical forms for dermatological application*, guidelines have been defined for the selection and specific formulation of excipients for ointments according to the therapeutic response through studies on the skin absorption rate and duration of the effect. These tests have been followed by the *in vivo* application of ointments with excipients of different nature and composition [1], then by identifying the correlation of the *in vivo* results with those of the *in vitro* release test and *in vitro* simulated absorption test [2]. On the basis of the acquired data, a new aspect of fractal analysis applied to the technology was also developed by trying to find the presence of a fractal pattern in the microscopic image of the emulsions and to detect any changes in the fractal dimension caused by the loss of stability of the preparation [3].

Studies that had the goal to verify the availability of drugs from ointments depending on the gelling conditions and their subsequent mechanical treatment [4], and how the consequent rheological differences could also influence the *in vivo* availability of a drug [5,6], have shown that, during the preparation of ointments, relative to the needs of the manufacturing and packaging plant, different operating conditions can be alternatively chosen without compromising the *in vivo* availability of the drug [7].

With regard to *suppository dosage form*, the effect produced by soluble, water-soluble, insoluble but swellable in water auxiliary agents in the lipophilic excipient on the release rate of drugs of a different nature was studied. Subsequently, prolonged-release suppositories, formulated with synthetic and semisynthetic polymeric substances, as well as with coupling techniques of different types of excipients, were tested [8]. Therefore, studies have been developed on the design of *in vitro* and *ex vivo* models of the rectal compartment [9,10].

With respect to studies on the *quality assessment of medical devices*, research has been orientated towards the development and application of innovative techniques suitable for the control of materials designed for human contact or for the containment of substances and/or preparations for therapeutic use. Taking into account the important role assumed by the control of the surface properties of materials for biomedical applications, an experimental method has been developed to control the quality of medical devices made of plastic material such as bags for total parenteral nutrition (TPN), perfusion containers, and the urethral catheter. The project was developed using two reproducible high-resolution techniques: tensiometry and atomic force microscopy [11, 12].

The research on oral dosage forms is focused on the development of a roadmap that facilitates the development of an efficient and robust granulation process, reducing time and costs, and pursuing the principles of quality by design. Since the evolution of the granulation process is closely related to the rheological properties of the wet mass and to the experimental conditions, a careful characterization of the properties of the raw materials and the rheology of the wet mass can help in the process development and scale-up. Studies carried out in this area have permitted to develop protocols based on the rheological characterization, able to predict the evolution of the granulation process and help the formulation development [13].

The line of research," Nature *inspired advanced drug delivery systems*" developed a series of advanced drug delivery systems based on materials/excipients available in nature. The silica sol-gel production technology has been optimized to generate carriers for the controlled or accelerated release of bioactive molecules of different nature, such as low-molecular-weight compounds and proteins have been developed [14].

In addition, by exploiting the natural high affinity interaction of the egg white protein avidin with nucleic acids, an innovative nanoparticle system was developed, and its usefulness, as an in *in vitro* and *in vivo* diagnostic tool and as a carrier for targeted drug delivery was demonstrated [15].

The research has developed *advanced surface tensiometry techniques* and models functional to the development of pharmaceutical formulations. Surface tensiometry characterization of pharmaceutical formulations has led to the development of the "*Rossi Number"*, a new pure number capable of characterizing the release and permeation properties in a rapid and non-invasive manner. The "*Rossi Number*" was accepted by the American Chemical Society. At the

same time, a new concept of the Integrated Analytical Approach was developed, capable of integrating surface tensiometry, rheological, and chemical data. [16].

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Tuesday, September 12
Session 7

Room A - Sala Tergeste

Chairpersons *Paolo Giunchedi – Silvia Arpicco*

16.55-17.20 Electrospun nanofiber matrices: a platform for soft tissue repair, regeneration and drug delivery

Bice Conti, University of Pavia

17.20-17.45 Can lipid-based nanosystems be a tool to overcome skin barrier?

Rita Cortesi, University of Ferrara

17.45-18.10 An evolution of mucoadhesive drug carriers based on chitosan and cyclodextrin derivatives

Anna Maria Piras, University of Pisa

18.10-18.25 *Journal of Controlled Release*

18.25-19.00 *Poster session*

20:00 Social Dinner

1973-2023 – 50th ADRITELF Anniversary

IV Convegno della divisione di Tecnologia Farmaceutica - SCI

Trieste September 11-13, 2023

ELECTROSPUN NANOFIBER MATRICES: A PLATFORM FOR SOFT TISSUE REPAIR, REGENERATION AND DRUG DELIVERY

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The ideal approach in tissue regeneration and repair should be minimal invasive, therefore not involving autologous tissue grafts. In these terms tissue engineering offers the opportunity of polymer devices promoting cell proliferation, and able to guide tissue regeneration whose principal objective is to recapitulate extracellular matrix (ECM) function in a temporarily coordinated and spatially organizedstructure [1].

The global market for tissue engineering has been estimated at $2,374 million in 2019, and is forecasted to growth to $6,815 million by 2027, with a 14.2% CAGR from 2020 to 2027. Based on type, the market is categorized as synthetic scaffolding material, bio- based scaffolding material, and others. The synthetic scaffold material segment is expected to experiencesignificant growth due to its effectiveness compared to other materials of natural origin. Synthetic materials are preferable to other materials in that they provide better outcomes [2].

Electrospinning, a key enabling technology (KET) as defined by the European Commission, is currently one of the best techniques allowing membranes prototyping composed of continuous fibres with diameters down to a few nanometres. The technique can be applied to synthetic and natural polymers in order to obtain 3D porous solid membranes to be used as scaffolds or formulations for tissue regeneration, tissue repairand/or drug delivery [3]. The nanofibre membranes show high surface-to-volume ratio, high porosity, and variable pore-size distribution, that give the membranesunique characteristics and can be tuned according to the intended scaffold application, by setting suitable electrospinning process parameter by a design of experiment (DoE) approach [4]. Moreover, polymer composition is of utmost importance in order to meet the intended scaffold mechanical and biological properties. Biodegradable biocompatible polymers such as polylactide (PLA) and its copolymers polylactide-co-glycolide (PLGA), polylactide-co-polycaprolacton (PLA-PCL) have been thoroughly studied by the authors to obtain electrospun nanofiber matrices suitable to be applied as temporary scaffolds for soft tissues repair and regeneration (**Figure 1**) with various focuses. The author's therapeutic focuses are ontubular organs such as oesophagus and small vascular vessels, and severe wounds (**Figure 1 A – C**).

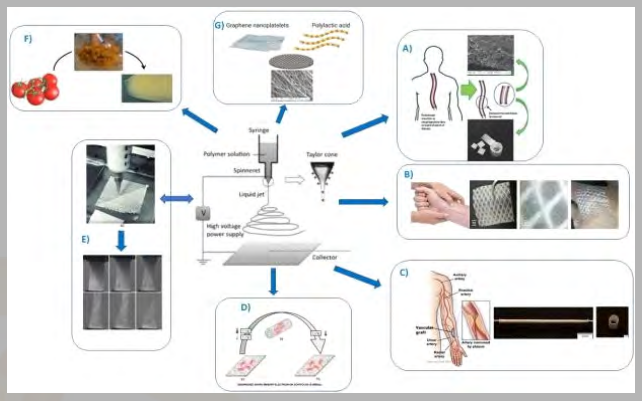

Figure 1. Recently developed and on-going projects involving electrospinning applied to tissue engineering, repair and drug delivery: A) PLA-PCL full thicknessscaffolds for oesophagus regeneration; B) Advanced fibrous textured dressing to treat severe wounds; C) Tobramycin supplemented small-diameter vascular grafts for local antibiotic delivery; D) Shape memory engineered scaffold(SMES) for mini-invasive surgery; E) 3D printing combined to electrospinning; F) Electrospun cutin-based polymer (eCUT); G) Graphene nanoplatelets-based textured polymeric fibrous fabrics.

The technical and formulation aspects in investigation encompass combination of electrospinning with 3D printing techniques, evaluation of composite materials such as PLA/graphene nanoplatlets, newly synthesized polymers (polyglycerolsebacate, PGS), newly processed natural polymers such as cutin-based polymer (eCUT), a secondary raw material derived from a recycled tomato waste [1, 3–14]. Two studies led to file patents, i.e., texturized dressing production and its application in topical delivery, and applicationof shape memory polymer properties in the design of engineered scaffolds [WO2021/064673-A1; PCT/IB2022/056647–P2031PC]. The main manufacturing technique studied is the electrospinning, but each topic involves a specific design study addressed to fit the optimal formulation according tothe specific needing. Moreover, thorough biological characterization is performed to evaluate and confirm the suitability of prototypes to *in vivo* application. Eventually, the prototypes from the most advanced projects developed (**Figure 1 A, B**) are being *in vivo* evaluated.

The ongoing project on Small-Diameter Vascular Grafts (SDVG) (**Figure 1C**) is an example of research

project combining tissue engineering with drug delivery, with the aim to achieve effective local antibiotic delivery that can be advantageous in decreasing side effects due to systemic administration, and antimicrobial resistance (AMR). The focus is on peripheral artery occlusive disease, an emerging cardiovascular disease characterized by the blockage of blood vessels in the limbs and associated with dysfunction, gangrene, amputation, and a high mortality risk. Possible treatments involve by-pass surgery using autologous vessel grafts, because of the lack of suitable synthetic small-diameter vascular prosthesis. One to five percent of patients experience vascular graft infection, with a high risk of haemorrhage, spreading of the infection, amputation and even death. In this work, an infection-proof vascular graft prototype was designed and manufactured by electrospinning 12.5% w/v poly-L-lactic-co-glycolic acid solution in 75% v/v dichloromethane, 23.8% v/v dimethylformamide and 1.2% v/v water, loaded with 0.2% w/w Tobramycin (TOB, Prototype1). Polymer and TOB concentrations were selected after viscosity and surface tension and after HPLC-UV encapsulation efficiency (EE%) evaluation, respectively. The final drug loaded prototype had an EE% of 95.58% ± 3.14%, with smooth fibres in the nanometer range and good porosity (**Figure 2 B,C**); graft wall thickness was 291±20.82 µm and its internal diameter was 2.61±0.05 mm.

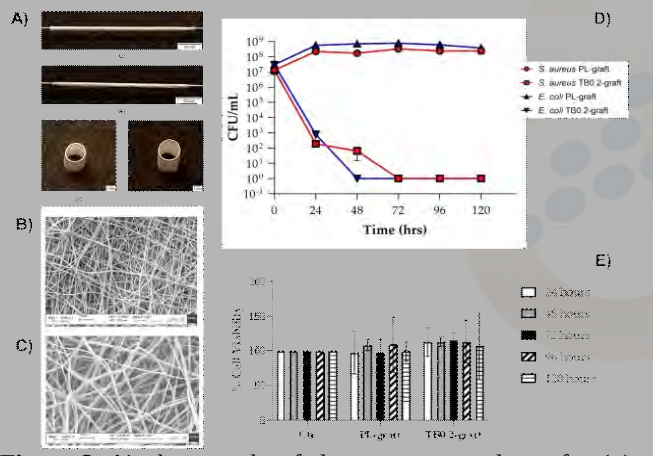

Figure 2. A) photographs of electrospun vascular grafts: (**a**) PL-graft and (**b**) Prototype1-TOB0.2-graft on collector; (**c**) PL-graft and (**d**) Prototype1-TOB0.2-graft horizontal sections; B) and C) SEM of PL and Prototype1-TOB0.2 graft; D) Bacterial population expressed as CFU/mL of S. aureus and E. coli, when placed in contact with PL-grafts and Prototype1-TOB0.2-grafts for 5 days; E) Cell viability expressed as % of viable NHDF after contact with extracts of PLgraft and Prototype1-TOB0.2-graft, in comparison with the 100% cell viability of control (Ctr).

The graft's antimicrobic activity evaluation through time-kill assays demonstrated a significant and strong antibacterial activity prolonged over 5 days against *Staphylococcus aureus* and *Escherichia coli*; indirect cell viability assay on Normal Human Dermal Fibroblasts (NHDF) confirmed the cytocompatibility of the grafts (**Figure 2 D, E).**

To improve the mechanical properties of the TOB-loaded grafts, a comparison between prototypes obtained from 12.5% (Prototype1) and 10% (Prototype2) w/v PLGA solution was performed: the significant smaller fibres and graft wall thickness of Prototype2 positively affected the elastic modulus in both circumferential and longitudinal directions (31.1 MPa and 6.9 MPa, respectively), resulting closer to that of saphenous vein, and an improvement of the graft's compliancy was also obtained. Moreover, *in vitro* drug release studies performed in PBS pH 7.4 at 37°C on both prototypes for two weeks, demonstrated differences on TOB release kinetics which resulted to be described by the Gompertz model and Peppas- Sahlin model respectively for Prototype1 and Prototype2. These results highlight once more that the formulation step has a pivotal role in ensuring the performance and effectiveness of drug eluting advanced medical devices. SDVG patency is a main issue in their *in vivo* application, and it is related to SDVG composition, architecture, and mechanical properties. Further studies are in progress to assess the SDVG hemocompatibility.

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CAN LIPID-BASED NANOSYSTEMS BE A TOOL TO OVERCOME SKIN BARRIER?

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It is well known that skin is an important body barrier towards external chemical, mechanical, physical and microbial stresses, resulting in a protection against pathogens and water loss [1]. Nevertheless, skin maybe affected by many disorders, such as rashes, infectionof different nature (i.e. viral, bacterial, fungal and parasitic), injuries caused by cut or burn, and tumours [2]. The treatment of skin disorders can be done with topical applications of drugs on the action site. In this way the drug is less involved in causing systemic side effects than with systemic application, since the drug barely reaches the systemic circulation. When a high concentration of a drug is required in the affected area, intra-dermal injection may be considered, but the use of more appropriate administration strategies to treat systemic diseases may be more beneficial and effective [3]. The success of topical treatments may depend on the type of vehicle used to deliver the drug to the skin, but also on the method of administration

With the advent of nanotechnologies, it has been possible to develop more efficient drug deliverysystems and the production of nanoscale material for technological applications, such as lipid-based nanosystems, has been investigated. Particularly, lipid-based nanoparticles, such as liposomes, ethosomes, transferosomes, solid lipid nanoparticles (SLN), nanostructured lipid carriers, (NLC) cubosomes and monoolein aqueous dispersions (MAD) (whose morphology are summarized in Figure 1), have been proposed for cutaneous application reaching in some cases the market or clinical trials.

These biocompatible lipid-based nanosystems allow the dissolution of lipophilic compounds and are able to control the release of carried drug possibly reducing side effects. Furthermore, the structural similarity between the lipids composing the nanosystems withthose of the skin represents one of the main advantages of lipid-based nanosystems allowing the interaction between the nanosystem matrix and the stratum corneum. With regard to cutaneous administration, the understanding of the nanoparticle interactions with the skin structures is of paramount importance in order to obtain information about drug delivery through the skin.

In particular, the application of active molecules with antioxidant activity has been considered. The possibility to employ a biocompatible formulation, consisting of lipids as the matrix and herbal extract as the drug, assures to the consumer a "green" approach, as well as almost the absence of adverse effects once applied on the skin. For instance, SLN and NLC have been proposed for the cutaneous application of essential oils, suggesting their use in inflammation phenomena, wound healing, fungal infections and skin aging. Furthermore, glyceryl monoolein based systems containing curcumin or saffron derivatives have been demonstrated suitable for the treatment of inflamed skin or wounds.

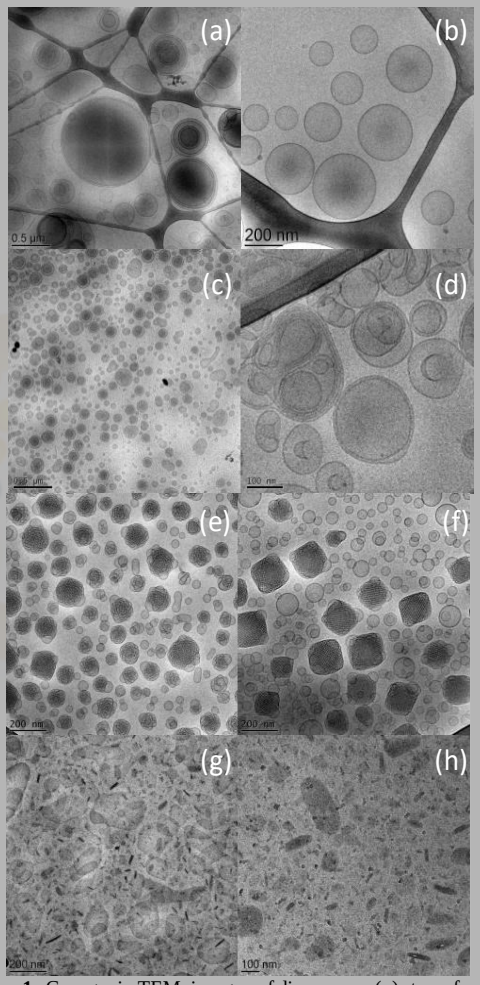

Figure 1. Cryogenic-TEM images of liposomes (a), transferosomes (b), niosomes (c), ethosomes (d), MAD (e), cubosomes (f), SLN (g) and NLC (h).

Overall, from our studies it seems that the cutaneous application of lipid nanosystems allows a deep interaction between lipid matrix and skin strata, promoting a prolonged release and efficacy of the loaded natural molecules. Anyway, many research studies have still to be performed and discussed toobtain information about the interaction of the nanosystem loads with the skin.

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AN EVOLUTION OF MUCOADHESIVE DRUG CARRIERS BASED ON CHITOSAN AND CYCLODEXTRIN DERIVATIVES

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Università di Pisa, Dipartimento di Farmacia

Progress in pharmaceutical technology has resulted in the selection and development of new functional excipients for drug delivery formulations. Excipients and relevant formulations, including in-situ gel forming systems and nanocarriers, can provide mucoadhesion, permeability enhancement, solubilisation and stabilising features, which are mainly devoted to improving drug bioavailability.

Since more than two decades, part of the pharmaceutical technology research activities at the University of Pisa have been focused on macromolecular excipients, mainly as semi-synthetic chitosan and cyclodextrin derivatives, as well as their use in nanocarrier formulations for oral, ocular, and more recently pulmonary administration routes.

Mucoadhesive polymers can be distinguished into first- and second-generation derivatives. First generation of mucoadhesive polymers are able to form non-covalent bonds such as hydrogen bonds, set electrostatic interactions and physical interpenetration to the mucus layer [1], whereas their second generation covalently binds to mucus glycoproteins via the reversible formation of disulfide bonds [2, 3].

Quaternary ammonium chitosan derivatives (QA-Ch) have been mostly investigated, demonstrating improved features compared to native chitosan. Those features include mucoadhesion and permeation enhancement, i.e through interaction with cellular tight junctions, as well as strengthened antimicrobial and antibiofilm properties [4]. QA-Ch derivatives were applied also in the assembly of nanoparticles (NP), preserving the mucoadhesion properties [5] and appearing particularly useful for the delivery of labile actives.

Several multifunctional derivatives have followed, containing both quaternary ammonium and thiol moieties QA-Ch-SH. Nanoparticles (NP) based on QA-Ch-SH polymers have been applied to prolong drug pre-corneal residence time, compared to QA-Ch NP. Soon after, thermosensitive hydrogels containing QA-Ch-SH and sulfobutyl chitosan NP demonstrated prolonged 5-fluorouracil ocular residence and increased ocular bioavailability [6]. A second generation of thiomers, with S-protected moieties (QA-Ch-S-pro) have been investigated to avoid SH stable oxidation, improved handling and an easily reversible redox interaction with mucins. Under oral administration, the differences in NP transport ratio through mucus and adhesion to excised rat intestinal mucosa suggested a higher mucoadhesivity of QA-Ch-S-pro NP compared to QA-Ch based NP. This directly influenced drug oral bioavailability in rats [7]. Both NP types improved the stability of labile compounds, i.e. natural extracts, and the permeation parameters, with respect to the control. In addition, the thiol derivative conferred better protection from the oxidative stress in *in vitro* simulated inflammatory conditions [8].

In the last years, thiolated cyclodextrins (CDs) were obtained by microwave assisted thiolation of the oligomeric backbone of different types of CDs. The derivatives combine the solubilization capability of the pristine cyclodextrins with the thiol related mucoadhesion, preserving also the excellent biocompatibility of the native compounds [3, 9]. Moreover, the thiolated cyclodextrins spontaneously formed nanoaggregates at low concentrations. The presence of thiol has then allowed the covalent stabilization of the nanoaggregates and their biopharmaceutical properties, related to the pulmonary administration route are currently under investigation.

To conclude, several methyl-β-cyclodextrin grafted to quaternary ammonium chitosans have also been investigated, performing as permeation enhancers, and preserving the complexing capability of bound cyclodextrins. The observed abilities are attributed to a synergistic cooperation of cyclodextrin and polymer, occurring only when the former is covalently linked to the latter [10, 11]. These derivatives have been investigated either as macromolecular carrier as well as nanoparticulate drug delivery system, for the administration of poorly soluble corticosteroids [10-12] or labile molecules, i.e. Dalargin neuropeptide and bergamot essential oil [13-15]. *In vitro* and *ex-vivo* studies revealed a stronger mucoadhesivity of the macromolecular complex, resulting in a slower transport through mucus with respect to NP, and a faster drug permeation through excised rat intestine. NP have a greater ability of preserving the labile molecules and resulted effective in crossing the *in vitro* models of epithelial and endothelial barriers.

The lively research activity, performed on mucoadhesive carriers, have contributed to the scientific debate on mucoadhesive *vs.* mucopenetrating carriers, evidencing the ocular and oral bioavailability enhancement generally achieved with mucoadhesive chitosan and cyclodextrin derivatives. The most challenging next step is a selective cellular targeting… working is in progress.

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Tuesday, September 12
Session 8

Room B - Sala Zodiaco

Chairpersons *Carmelo Puglia – Anna Rita Bilia*

16.55-17.20 When the pharmaceutical technology meets regulatory science
Paola Minghetti, University of Milan

17.20-17.45 Pharmaceutical technology platforms for the development of tailored paediatric dosage forms
Nunzio Denora, University of Bari "Aldo Moro"

17.45-18.10 The versatile role of cyclodextrins in drug delivery
Francesca Maestrelli, University of Florence

18.10-18.25 *Journal of Controlled Release*

18.25-19.00 *Poster session*

20:00 Social Dinner

1973-2023 – 50th ADRITELF Anniversary

IV Convegno della divisione di Tecnologia Farmaceutica - SCI

Trieste September 11-13, 2023

WHEN THE PHARMACEUTICAL TECHNOLOGY MEETS REGULATORY SCIENCE

Paola Minghetti, Francesco Cilurzo, Antonella Casiraghi, Francesca Selmin, Chiara Gennari, Silvia Franzè, Umberto M. Musazzi, Paolo Rocco

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Introduction

Research in pharmaceutical technology can have a high impact in improving the performance of medicinal products containing both new and old drug substances. Indeed, the same Active Pharmaceutical Ingredient (API) can be formulated in pharmaceutical forms or administered through route of administrations that are innovative from the technological or biopharmaceutical point of view and capable to improve clinical outcomes, by modifying pharmacokinetics and/or biodistribution, hence safety and efficacy, as well as patient adherence to therapy. Advancements in the technological field may require a modification or adaptation of existing legislative acts and regulatory guidelines, in order to maintain the level of protection of public health that is generally guaranteed by the pharmaceutical legislation. This establishes a strong link between pharmaceutical research and regulatory science, as it is the duty of pharmaceutical researchers, together with regulatory experts, to foster innovation in regulation by assessing new technologies and related requirements, as well as determining the Critical Quality Attributes (CQAs) of the new products and their risk-benefit balance.

In our research group the interest has been focusing to study the mutual interconnections between these two aspects: drug delivery systems and regulatory sciences. Indeed, this can be traced back to the study of (trans)dermal patches, in particular with the aim to relate the relevance of adhesive properties to efficacy and safety and promote the introduction of assays for quality control purpose [1]. Meanwhile, an in-depth study on the development of copies of long-acting injectable (LAI) has been carrying out, from the formulation aspects - including sterilization - to the regulation aspects of this pharmaceutical form that is considered complex drugs [2].

The know-how acquired on the preparation and characterization of transdermal patches has led to the design of mucoadhesive dosage forms, that share the topic of the adhesion to biological tissues, and, subsequently, orodispersible films (ODF) that are produced with the same technologies. The knowledge gained through studies on the skin and pre-gastric mucosae penetration studies and LAI has driven the interest towards nanotechnology, focusing not only to the design, but also to the regulations to guarantee the safety of nanosystems [3].

Finally, as the industrial medicinal products cannot always meet the patient's needs, the research deals on the development of innovative technological solutions to be applied in compounding pharmacy setting.

Transdermal patches

The main focus in the development of transdermal patches was on the biopharmaceutical aspects related to the improvement of the drug skin permeation with the aim to assure therapeutic concentrations and minimize the patch surface. The most studied approaches included the use of chemical enhancers, which can present the issue of their diffusion in the adhesive matrix, or the design of stable supersaturated systems able to improve the drug thermodynamic activity. In recent years, we have addressed issues concerning the equivalence assessment of drug fluxes in *in vitro* skin permeation studies to support generic or abridged applications marketing authorizations or to manage dossier variations during the product cycle life. In particular, the impact of the inter-skin sample variability is the most debated point both for patches and semisolid preparations. Using formulation pairs (i.e. plaster vs. patches)containing propranolol, diclofenac or nitroglycerine, we defined the acceptability interval and number of replicates to be performed [4]. As an example, the equivalence of two propranolol patches (flux variability lower than 25%) can be assessed using six replicas and a confidence limit within the 0.8–1.25 range (α=0.05; power 90%). In contrast, the equivalence of diclofenac plasters, which exhibit a variability near 50%, can be demonstrated increasing the number of replicas (i.e., 20 skin samples) for each formulation and widening the acceptance range according to a statistical approach derived by bioequivalence studies performed in parallel.

Long-acting parenteral dosage forms

LAI are well-established dosage forms to control the drug release from weeks to months. In particular, polymer-based LAI are generally constituted of biocompatible and biodegradable PLGA, which have a consolidate safety profile as such and upon degradation. As LAI are defined as complex drug, the Regulatory Authorities require an in-depth knowledge on their biopharmaceutical properties to develop a generic equivalent.

First, sterilization by ionizing radiations is generally used for PLGA-based LAI, even if bond scission and crosslinking phenomena occur over time in a stochastic way. Moreover, the presence of oxygen during sterilization or the storage period can cause unpredictable variations in both the drug content and release kinetics [5]. Furthermore, the impact of radiations on the loaded drug or excipients has to be

evaluated case by case. As a matter of fact, PEG up to 30% acted as radiostabilized on ovalbumin, avoiding the formation of dimers; HPMC chains, being more sensitive to β-irradiation, were preferentially cleaved, reducing the detrimental effect on morphology, chemical, and physicochemical properties of methylprednisolone loaded microspheres. To solve this issue, we designed PLGA grafted to antioxidants (i.e., caffeic acid) stable upon irradiation which were able also to improve the encapsulation efficiency of hydrophilic drugs, such as fluvastatin [6].

Secondly, there are not compendial tests to assess the in vitro drug release, nor a unanimous consensus on the composition of the extra-cellular matrices that can be used to design biorelevant media. As an example, synovial fluids significantly differ health or disease state (i.e., the protein concentration) and this have an impact on the released of methylprednisolone from microsphere and PLGA degradation [7].

Orodispersibile films

The emerging interest towards a patient-centric design of pharmaceutical forms has driven the development of orodispersible films (ODF) for patients with swallowing issues. ODF are mono- or multilayer "strips" constituted by materials with good film-forming capabilities and produced mainly by solvent-casting. Considering the limited formulation space, drug dose and excipients' selection are critical for both the drug stability and ODF organoleptic and mechanical properties. Through the years, several technological platforms have been optimized in our laboratory and patented for healthcare applications. Innovative approaches have also been investigated to improve ODF biopharmaceutical properties: e.g., tuning drug release by the incorporation of microparticles up to 12% w/w of loading. By modulating ratio between free and microencapsulated drug (i.e., melatonin) in the same matrix, we demonstrated the feasibility to combine the immediate release with a sustained release over at least 5 hours [8]. In parallel, the research is currently dealing with the use of printing technologies (e.g., hot melt ram extrusion printing) to prepare ODF in small batches for personalized therapy [9]. Furthermore, it is matter of investigation the regulatory pathway to enable real-time batch release of the printed products which require online monitoring of the CQAs and other critical process parameters. Such aspects are strictly connected to the development and validation of non-destructive quality-control methodologies.

Nanotechnology based products

From a regulatory perspective, nanotechnology-based products fall in a wide range of regulatory classes depending on nanomaterial type, application, or industrial sector [3]. Moreover, there is a lack of routine, validated methods for the physio-chemical characterization. When the attention is moved towards nanotechnology-based products intended for topical administration, the situation becomes even more complicated since the existing guidelines were devised mainly for nanosystems intended for parenteral administration and no indications on their quality attributes for topical application are provided . The most studied drug delivery systems are deformable liposomes which, consisting of "fluid-state" lipids and softener agents, present a fluid and deformable membrane that should facilitate the crossing of stratum corneum. Recently, we designed a novel, dual carrier based on deformable liposomes technology, namely a drug-in-micelles-in-liposomes (DiMiL) system that showed improved stability and resulted very promising for the delivery through the skin of poorly permeable compounds and also fixed dose drug combinations [10]. For all these carriers, deformability is a critical attribute. Thus, a routine method capable of directly measuring the bilayer rigidity (σ) and vesicle deformation (K) of liposomes during the penetration through narrow pores (50 nm) by using a dynamometer was proposed. The method resulted reliable since σ and Young modulus values calculated by Atomic Force Microscopy perfectly correlated (R^2 = 0.935). TEM images taken on full-thickness human skin samples demonstrated the existence of a relationship between the deformability of liposomes and the depth of penetration of intact vesicles in the skin, confirming the relevance of this parameter in the design of liposomes to be applied on the skin [11].

Compounding medicinal products

There is a growing need to have available real world data to support post-marketed safety. As an example, during the mass vaccination against COVID-19, the stability of lipid nanoparticles in pre-drawn syringes was assessed since no changes were observed in after 5 h and upon transportation [12].

We provided experimental data to standardize the operative conditions for compounding magistral formulae to fulfil unmet medical needs, as in the case of flecainide oral solution. The optimized formulation, which solved the incompatibility issues, is under discussion for the inclusion in the European Paediatric Formulary [13]. In case of cannabis oily extract, the preparation method was optimized in terms of extraction time and temperature to maximize THC content [14].

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PHARMACEUTICAL TECHNOLOGY PLATFORMS FOR THE DEVELOPMENT OF TAILORED PAEDIATRIC DOSAGE FORMS

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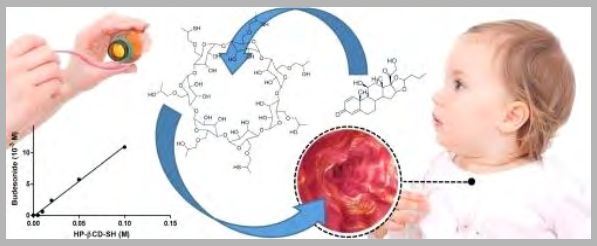
Thereafter the entry into force of the Paediatric Regulation in 2007 [1], the unit of Pharmaceutical Technology and Regulatory Affairs (Phartecolab) ofthe University of Bari Aldo Moro began to explore and develop paediatric dosage forms using innovative, lab-made and scalable platforms such as microfluidics, prilling technology and direct powder extrusion 3D printing to produce tailored products with features that meet the specific requirements of children. The development of appropriate drug formulations for the paediatric age still represents a challenge and an unmet need. In fact, more than 50% of medications on the market are not taken as advised and around one tenth of prescriptions for children are either off-label or unlicensed. To fill in the gap, legislative and regulatory acts have been issued to promote high quality research for paediatric medicines development and availability. Hence, when developing a paediatric formulation, it is important to consider that, for each of the five agegroups (Figure 1), the medication must be optimized considering differences in the physiology and anatomy of each age group, pharmacodynamics and pharmacokinetics, as well as issues related to therapy acceptability and handling.

Figure 1. Paediatric age groups.

Phartecolab has long been attentive to these paediatric needs and, thanks to the diverse expertise of its researchers on established and new formulation approaches, investigated and developed paediatric formulations, that could improve therapy outcomes by increasing patient adherence.

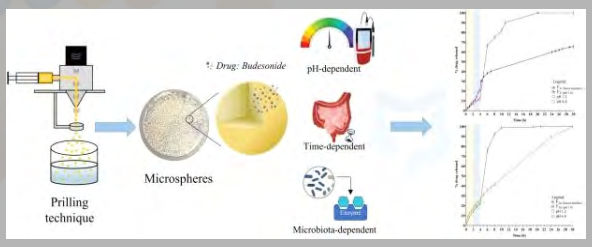
By partnering with private and public entities, several unmet paediatric needs have been examined in several studies regarding the development of tailored formulations for children. These studies focused on:

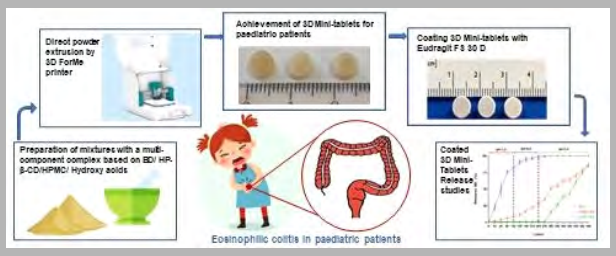
1) Solving biopharmaceutical problems (solubility and dissolution issues) with pharmaceutical technology-based strategies, especially using pristine or chemical modified cyclodextrins [2];



2) Reformulation of repurposed drugs for paediatricuse and particularly formulations for the treatment of paediatric rare diseases by using nano-delivery systems produced by microfluidics [3];

3) Design and development of oral formulations with high appeal in children such as micro-particulates and mini tablets through prilling/vibration techniques and direct powder extrusion 3D printing, respectively [4-5].





The achieved research journey will be summarized in this report through the presentation of some of the main case studies investigated by Phartecolab in recent years.

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THE VERSATILE ROLE OF CYCLODEXTRINS IN DRUG DELIVERY

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Cyclodextrins (CDs) are versatile excipients with an essential role in drug delivery, since they can form non-covalently bonded inclusion complexes with several drugs. These multifunctional excipients are able to increase solubility, dissolution rate, chemical stability, permeation and bioavailability of different drugs [1], [2]. However, some disadvantages, such as fast removal from the blood stream after in vivo administration, or possible exchange of the included drug moieties with other molecules endowed with greater affinity for the CD cavity, could reduce the CDs effectiveness as drug carriers. Strategies based on the addition of a proper third component [3], or on the combined use of CD complexation and loading of the complexed drug into different colloidal carriers [4] were successful in circumventing the above problems and join the relative benefits of all components. CDs have been employed to formulate a variety of both conventional and innovative drug delivery systems designed for several administration routes by virtue of their great formulation versatility and safety. For example, stable oral pediatric solutions of hydrochlorothiazide were obtained by the joined use of CDs and hydrophilic polymers; in vivo studies on rats showed that this formulation allowed a more pronounced and more reproducible diuretic effect than the corresponding suspension [5]. Conventional and immediate release tablets of flufenamic acid were obtained by grinding the drug with randomly methylated-β-CD, obtaining an improved and well reproducible dissolution performance, regardless of the different drug batch used [6]. The absorption enhancer properties of CDs can also be successfully exploited in drug delivery systems. Polymeric epichlorohydrin-β-CD resulted the best choice for binary systems with curcumin included in topical gels, allowing not only an increase of drug solubility and stability, but also of skin permeability [7]. Recently performed in vivo test demonstrated the potential of such a formulation for a topical anti-inflammatory treatment. CDs have been profitably applied also for improving intranasal drug delivery: randomly methylated β-CD demonstrated the ability to improve mucoadhesive strength, mucoadhesion time and release rate of clonazepam from nasal gels; moreover it was also able to reduce drug cytotoxicity and further improve its permeability through Caco-2 cells [8]. Several examples of applications of combined strategies simultaneously exploiting the use of CD complexation and of a suitable nano-carrier have been reported, highlighting the possible advantages achievable by such a dual strategy, depending on the CD-nanocarrier combination, and mainly resulting in enhanced performance of the delivery system and improved biopharmaceutical properties and therapeutic efficacy of drugs [4]. Our most recent work concerns the development of an epichlorohydrin-β-CD-based nanogel that resulted able to regulate and prolong ibuprofen topical release and promote its permeation through skin-simulating artificial membranes [9]. The effectiveness of a combined approach taking advantage at the same time of the CD solubilizing effect and the liposomes carrier function to improve the therapeutic efficacy drugs, has been well proved [10, 11]. The possibility to efficiently entrap curcumin as complex with 2-hydroxylpropyl-β-CD in-liposomes by a double- loading technique has been shown, demonstrating the enhanced therapeutic effect of this formulation in comparison with a solution in a model of osteoarthritis induced in rats [12]. Drug-in cyclodextrin-in solid lipid nanoparticles (SLN) or in nanostructured lipid carriers (NLC) were also favourably applied to develop oral pediatric formulations of hydrochlorothiazide [13], [14]. The drug coground product with hydroxylpropyl- β-CD allowed in both cases a higher entrapment efficacy and a sustained drug release, and in vivo studies on rats proved the superior therapeutic effectiveness of the formulations containing the drug as CD-complex, thus confirming the successfulness of the proposed approach.

New CD-based drug delivery systems are constantly being developed, and new possible applications are being explored, proving the great and continuous interest towards these excipients that amply proven to be a very useful and versatile tool for improving the drug formulation performance.

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Wednesday, September 13

Chairpersons *Paolo Caliceti*

9.00-9.40 Prof. Andreas Bernkop-Schnürch, University of Innsbruck
Lipid-based Nanocarriers for Oral peptide Drug Delivery: Hype or Hope?

Chairpersons *Ruggero Bettini*

9.40-10.20 Prof. Maria José Alonso, University of Santiago de Compostela
Advanced therapies and Personalized Medicine: The role of Pharmaceutical Nanotechnology

10.20-10.50 *Coffee break*

PLENARY LECTURE

Lipid-based Nanocarriers for Oral Peptide Delivery: Hype or Hope?

Andreas Bernkop-Schnürch

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Within recent years lipid-based nanocarriers such as solid lipid nanoparticles (SLN), nanostructured lipid carriers (NLC), w/o nanoemulsions and self-emulsifying drug delivery systems (SEDDS) have been discovered for oral administration of peptide drugs. Due to the formation of hydrophobic ion pairs even highly hydrophilic peptides can be incorporated in the lipophilic phase of these carrier systems. As lipid-based nanocarriers provide a protective effect towards gastrointestinal peptidases (I), exhibit high mucus permeating properties (II) and can interact with the absorption membrane favouring the systemic uptake of their payload (III), meanwhile for some peptides an oral bioavailability >10% can be reached. Future advances such as the use of dry reverse micelles instead of hydrophobic ion pairs or the design of charge converting lipid-based nanocarriers will result in even more efficient delivery systems.

Andreas Bernkop-Schnürch is chairman for Pharmaceutical Technology and Head of the Department of Pharmaceutical Technology at University of Innsbruck; founder and CSO of Thiomatrix Forschungs- Beratungs GmbH; founder of MucoBiomer GmbH (meanwhile part of the Croma Holding) and Green River Polymers GmbH. He pioneered various novel technologies such as thiolated polymers (thiomers), charge converting nanocarriers for mucosal drug delivery and advanced lipid-based formulations for oral peptide drug delivery. Andreas Bernkop-Schnürch has been awarded more than twenty international and national awards including the Houska Award 2007, Ernst Brandl Award 2015, Gattefossé North America Award 2017 and Phoenix Science Award 2022.

PLENARY LECTURE

"Advanced therapies and Personalized Medicine: The role of Pharmaceutical Nanotechnology"

Maria J. Alonso

CIMUS and IDIS Research Institutes and Faculty of Pharmacy

University of Santiago de Compostela, Spain

Pharmaceutical nanotechnology may contribute to the development of precision medicine by facilitating the access of anti-cancer drugs and their therapeutic target. Our laboratory has contributed to paving the way towards the targeted drug delivery of oncological drugs, notably biological drugs.

In the last decade we developed polymeric nanocapsules with the ability to target the cytotoxic drugs to the tumoral tissue and, also, to the lymphatics where metastatic cells may reside. In vivo studies performed with model drug docetaxel showed the potential of this technology for the reduction of tumor growth and elimination of the metastatic spreading.

Subsequently, we explored a variety of nanotechnologies for the **targeting of intracellular oncoproteins using mAbs**. The results obtained so far, are remarkable and show the capacity of MPN-mAb to target intracellular oncoproteins such as KRAS. More precisely, it was found that MPNs could transport mAb into the cytosol and facilitate its endosomal escape. These positive results correlated with the observed significant reduction of the tumor size/weight in several mice tumor models.

Finally, in the context of advanced therapies, we have recently developed new nanotechnologies for the targeted **delivery of RNA molecules**. The results have shown the possibility to silence the KRAS oncoprotein by delivering siRNA anti-KRAS in a pancreatic cancer model.

More information about these projects and associated publications can be found at:

http://www.usc.es/grupos/mjalonsolab/

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The following researchers have contributed to the projects: A. Lopez, D. Teijeiro, S. Anthiya, D. Torres

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María José Alonso is Professor of Pharmaceutical Technology at the University of Santiago de Compostela, Spain. She has worked at the University of Paris Sud and at the Massachusetts Institue of Technology (MIT). Her lab has pioneered numerous discoveries in the field of nanomedicine, notably in the design of nanotherapies for the treatment of cancer. She has been part of the "Power List" of the most influential researchers in the field of Biopharmaceuticals (*The Medicine Maker*) (H Index >100). She is a member of five Academies, among them the Royal Academy of Medicine of Belgium and the US National Academy of Medicine (NAM). She was also awarded with "Honoris Causa" doctorate by the University of Nottingham.

Wednesday, September 13
Session 9

Room A - Sala Tergeste

Chairpersons *Donato Cosco – Carlotta Marianecci*

10.50-11.15 The landscape of pharmaceutical technology in Turin University: focus on nanotechnology strategies

Roberta Cavalli, University of Turin

11.15-11.40 Antibody-drug conjugates and immunoliposomes against her2 positive cancers.

Gianfranco Pasut, University of Padua

11.40-12.05 Macro- micro- and nano drug delivery systems @ DIFARMA - University of Salerno

Pasquale Del Gaudio, University of Salerno

12.05-12.30 Molecularly Imprinted Synthetic Material Antibodies (MISMAs) as artificial counterpart of conventional antibodies in molecular recognition

Francesco Puoci, University of Calabria

12.30-14.30 Lunch

The landscape of pharmaceutical technology in Turin University: focus on nanotechnology strategies

Monica Argenziano[1], Silvia Arpicco[1], Francesca Baratta[1], Chiara Bastiancich[1], Luigi Battaglia[1], Paola Brusa[1], Roberta Cavalli[1], Daniela Chirio[1], Marina Gallarate[1], Paola Milla[1], Elena Peira[1], Anna Scomparin[1], Barbara Stella[1], Elena Ugazio[1]

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Nanotechnology plays a relevant role in the pharmaceutical/cosmetic field, and, thanks to the development of smart and intelligent drug delivery systems, it allows to achieve different goals.

The pharmaceutical technology sector in Turin University owns a widespread and established expertise in the preparation and characterization of conventional and innovative drug formulations by means of nano and/or microparticles, based upon polymer, lipid and inorganic matrixes. In particular, squalene and polymeric nanoparticles, solid lipid nanoparticles, liposomes, polymer-drug conjugates, cyclodextrin-based nanoparticles, nanocrystals, nanovesicle-based systems (i.e. nanobubbles, nanocapsules), nano and microemulsion are engineered for pharmaceutical and/or cosmetic applications. Moreover, such research has also been focused on the development of targeted nanosystems, by using multi-pronged strategies Figure 1. [1-2].

Of note, such formulative approaches are all drug-driven. That is, first, formulation strategies are developed owing to the physico-chemical features of the drug/functional ingredient to be loaded within the micro/nanosystems, including hydro/lipophilicity, molecular weight, chemical and thermal stability; second, size and surface properties of micro/nanosystems are optimized, in order to enable them to deliver their cargo to its specific target site; third, micro/nanosystem composite structureis designed in order to enhance the drug solubility and/or to achieve its sustained release.

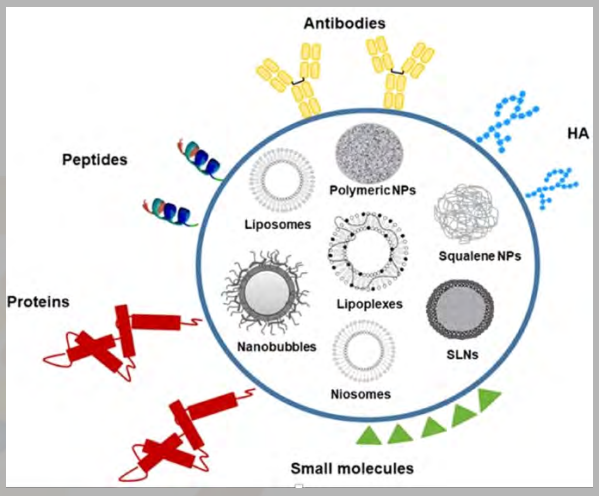

Figure 1. Schematic representation of most common actively targeted nanocarriers developed by
Italian Technology research groups using ligand-based strategies over the last 10 years [2].

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ANTIBODY-DRUG CONJUGATES AND IMMUNOLIPOSOMES AGAINST HER2 POSITIVE CANCERS

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Nanomedicines have evolved into a variety of forms including, for example, nanoparticles, liposomes, micelles, nanoemulsions, polymer-protein/drug conjugates, dendrimers, nanocrystals, polymeric vesicles, antibody-drug conjugates, nanogels, nanotubes, some already approved for diagnostic and/or therapeutic use. Since Doxil, a PEGylated liposome delivering doxorubicin, was first approved by the FDA in 1995, nanomedicine research has been taking giant steps forward although the pathway to success has not been entirely fulfilled, and even today some may argue that it has not fully lived up to its original promise to revolutionize therapy and diagnosis [1]. A nanomedicine project usually starts from analyzing the literature and comparing the pros and cons of a drug delivery system to another based on data commonly produced by different studies/papers and obtained from experiments performed under different conditions and variables. This makes the comparison unreliable and uncertain. There are not systematic studies comparing face-to-face the development and efficacy of two nanomedicines. Here we investigated the development, and the in vitro and in vivo characterization of two targeted nanomedicines, an antibody-drug conjugate (ADC) and a targeted drug loaded liposome, two formats that have already reached the clinical use. The aim was to elucidate the real benefits and eventual limitations of each approach for a future application by a direct comparison. We fixed a common point between the two nanomedicines: the same target, the human epidermal growth factor receptor 2 (HER2) that is mainly overexpressed in 20- 30% of invasive breast and ovarian carcinomas [2]. Consequently, the same antibody, trastuzumab (Trz), has been exploited for achieving the HER2-targeting. The delivered drug among the two approaches is different owing to the peculiar characteristic of each nanomedicine: doxorubicin (Dox) has been used for the immunoliposome and mertansine (DM1) for the ADC. DM1 is toxin already used for the clinical approved Kadcyla, it has a subnanomolar IC50 that ensures the preparation of an effective ADC. Owing to the limited number of drug molecules that can be coupled per mAb unit, in most cases around 4, the use of Dox would have generated an unactive ADC, as already demonstrated. Differently, Dox is already used for liposomes (Doxil), since in this case a too potent drug would yield issues of toxicity because the loading capacity of liposomes is higher than that of ADCs. In both cases, we sought introduce an innovation with respect to the state of the art: 1) for the ADC we developed new PEG-based linkers to allow increasing the common drug/mAb ratio (DAR) from 4 to 8, thus obtaining more potent ADCs but preventing the risk of aggregation due to the high hydrophobicity of the drug;

2) for the immunoliposomes, we used the Fab' of trastuzumab instead of the full mAb to prevent a great increase of liposome's size and favoring the orientation of the Fab' on the surface of the liposomes.

The prepared ADCs were: 1) VAG4 and VAG8, 2) CP4 and CP8, characterized by a different drug / antibody ratio (DAR), namely 4 or 8, and a different amino acidic linker in the spacer that ensures the drug release after cell internalization of the ADC (VAG = Val-Ala-Gly and CP = Phe-Gly, with the structure drug-linker(PEG)-mAb). The spacer joining Trz and the drug contains a pendant PEG oligomer to counterbalance the hydrophobicity of the drug. The immunoliposomes were: 1) Dox loaded stealth liposomes (SL), having a PEG5kDa-DSPE shielding, 2) Dox loaded stealth Trz Fab'-immunoliposomes (SIL), similar to SL but also modified on the surface with the Fab'-PEG-DSPE, and 3) Dox loaded super stealth Trz Fab'-immunoliposomes (SSIL2), in which the PEG is anchored to the liposome through 2 DSPE molecules (PEG5kDa-(DSPE)2) [3] and the targeting is achieved through the Fab'-PEG-(DSPE)2.

After the full characterization of the testing compounds and the determination of their pharmacokinetic parameters, the same were tested in vitro against SKOV3 (HER2+), SK-BR3 (HER2+) and MDA-321
(HER2-) cells. The targeted compounds showed improved activity on HER2+ cell lines, in fact the IC50 values could be calculated only in HER2+ cells. The IC50 values of immunoliposomes tested in SK-BR3 cells were 4.475 ± 0.429 μM and 1.001 ± 0.104 μM (Dox equiv.), for SIL and SSIL2, respectively. These values are significantly lower than that calculated for SL (7.656 ± 1.187 μM). In SKOV3 cells, IC50 were
1.285 ± 0.653 μM and 1.182 ± 0.985 μM (Dox equiv.) for SIL and SSIL2, respectively, lower than 10.65 ±
1.34 μM calculated for the untargeted formulation SL. The IC50 of ADCs with DAR 8 were similar to that of Kadcyla in SK-BR3 cells (1.564 ± 0.124 ng/ml and 1.525 ± 0.209 ng/ml for VAG8 and CP8, respectively), whereas in SKOV3 cells VAG8 was significantly more active in vitro than CP8 (IC50 = 2.419 ± 1.235 ng/ml vs 41.61 ± 5.636 ng/ml). This observation is in accordance with the expression of cathepsin B, a lysosomal enzyme responsible for the cleavage of the binding between the mAb and DM-1, which is necessary for cytotoxic activity. Cathepsin B expression is significantly higher in SK-BR3.

The in vivo potential of the compounds was tested in a mouse SKOV3 xenograft model. The high DAR ADCs

VAG8 and CP8 were administered at the dose of 2.5 mg/kg every 15 days while the immunoliposomes were administered at the dose of 5 mg/kg (Dox equiv.) every 5 days.
The ADCs showed the strongest activity, with a significant tumour reduction and an improved animal survival. Unexpectedly, the in vivo activity of CP8 was similar to that of VAG8. The immunoliposomes were more effective and better tolerated with respect the non-targeted liposomes (SL).
We also characterized the effect of ADCs and immunoliposomes on the immune tumor microenvironment, focusing on innate immune cells (macrophages and natural killer cells), since the use of an immunodeficient mouse (SCID), lacking adaptive immunity, was mandatory to permit the growth of human HER2+ expressing cells. To this aim, tumors were collected at sacrifice after 10 days of treatment and the immunophenotyping was performed by flow cytometry, after the set-up of a gating strategy allowing the identification of total and exhausted (protumoral) NK cells, and M1 (antitumoral) and M2 (protumoral) macrophages. We demonstrated that ADCs had negligible effect on macrophage phenotype, whereas significantly reduced the ratio between exhausted NK/NK cells. On the contrary, immunoliposomes were able to reduce significant the protumoral M2 macrophages in the tumoral tissue with respect to vehicle-treated control mice. Only SL and SIL also significantly reduced the ratio between exhausted NK/NK cells.

Overall, from the face-to-face comparison between immunoliposomes and ADCs, designed to target the same antigen (HER2), interesting conclusions can be drawn. From the preparation point of view, startingfrom the same mAb the level of complexity for the synthesis/formulation step and for the fine characterization, immunoliposomes are more complicated than ADCs and likely more expensive. Then, the specific features of ADCs ensure a prolonged pharmacokinetics, in fact their elimination half-livesare in the order of days (8-12 days) with respect to the hours of immunoliposomes (19-35 h). Furthermore, thanks to the more potent drug, DM1, delivered by ADCs they showed stronger in vitro/in vivo activities. Interestingly, given their efficacy, immunoliposomes and ADC showed a differential peculiar effect on the immune tumor microenvironment, suggesting that the use of a different nanomedicine can trigger different immune responses, although we cannot exclude that this can be correlated to the different drug delivered. Further studies are needed to confirm the clinical relevance of these findings, with the final aim of improving the personalization of cancer therapies.

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MACRO- MICRO- and NANO DRUG DELIVERY SYSTEMS @ DIFARMA UNIVERSITY OF SALERNO

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Università degli Studi di Salerno, Dipartimento di Farmacia

Several factors, including specific administration route, system components, size, zeta potential, drug loading and encapsulation efficiency, release kinetic, and biomolecule activity, as well as manufacturing technology, must be taken into consideration to develop a clinically successful drug delivery system. Moreover, the use of genomics and new diagnostic leads to identify patients' needs and predictable response, promoting the use of targeted treatments and personalised medicine [1].

Over the years, microparticulate and nanoparticulate drug delivery systems, as well as customizable dosages and constructs composed of biopolymers have been explored to deliver different active ingredients in a site-specific and controlled way [2]. When formulated with appropriate excipients, polymers and technologies they are able to protect liable biomolecules from *in vitro* and *in vivo* degradation, enhancing stability, and/or providing an increased surface to volume ratio, and in some cases enabling targeted release to a specific site, hence an improvement in bioavailability [3].

Polysaccharide based microparticles (MPs) and nanoparticles (NPs)

Wound healing is a physiological process able to maintain the integrity of skin after accidental or intent trauma which include partially overlapping phases, (homeostasis, inflammation, proliferation and remodelling). Excessive wound healing or chronic wound may impair this normal function. A large number of drug delivery systems and medical devices have been provided but wound healing remains an increasingly clinical problem due to an aging population, incidence of diabetes, atherosclerosis, and venous insufficiency. Our primary focus was to develop ppolysaccharide based microparticles (MPs) and nanoparticles (NPs) as an *in situ* gelling powders able to conform to wounds with irregular shape and depth, while promoting the controlled release of encapsulated APIs. Alginate-pectin-chitosan blends loaded with antimicrobial drugs, healing agents, peptidomimetics and nanocomposites, have been widely investigated able [4-5]. Spray drying and nano spray drying technologies have been optimized to manufacture particles with different size (few micrometers-hundreds nanometers). The systems are able to move from dry powder to hydrogel in less than 1 min, forming a transparent layer over the wound protecting while promoting transpiration (water vapour transmission rate between 93 and 102 g/m^{2}h), and avoiding wound dehydration or occlusion. Powders have shown no pro-inflammatory activity in terms of IL-6 and TNF-α levels, whereas they induce the release of IL-8 from the human keratinocytes that stimulates the wound healing process. APIs release is characterized by a burst effect related to particles hydration kinetic and polymers ratio, followed by a prolonged release, very useful to prevent infection spreading or to promote healing process over time.

According to the emerging topic of circular economy, utilization, recovery and re-use of materials from agro-food industry, part of our work on polysaccharide based micro- and nano-particles have been also dedicated at developing delivery systems tailored for botanical derivatives. Waste and by-products still contain bioactive ingredients, such as phenolic compounds, glycosides and terpenoids but also materials and bulking agents to produce topical emulsion, edible films, and oral dosage forms. The development of new botanical-based formulation involves several steps, including production of the extracts, chemical and biological characterization and design and control of the final dosage forms. Innovative and non-conventional extraction technique (ultrasound, supercritical fluids, and pressurized liquid extraction) led us to recovery of materials in high yield while minimizing the use of organic solvents. Quality control of the obtained raw materials (API and excipients) and finished products was based for purity and safety on advanced techniques such as UHPLC-UV-HRMS and 1D- and 2D-NMR and on in vitro assays (cytocompatibility, viability etc). Monitoring is completed by bioactivity tests (antioxidant, anti-inflammatory, antimicrobial, and anticancer). Due to the common organoleptic (sticky texture, pungent odor, and unpleasant taste), and biopharmaceutical characteristics (low stability and solubility in aqueous biological fluids), botanical-based materials, the development step of a formulation is very challenging. Many topical and oral drug dosage forms, food supplements and nutraceutic formulation were developed [6-7]

Polysaccharide based aerogels in form of bead for tissue regeneration have been developed by supercritical based technologies, with hemostatic and regenerative properties. Aerogels as nanostructured materials with up to virtually 100% overall porosity in the mesoporous range and with full pore interconnectivity are ideal substrates to promote cell growth. The optimization of processing parameters as well as the use of carbohydrate polymers has led to the development of core-shell aerogel particles loaded with different APIs [8]. The aerogels could easily move into a hydrogel blocking bleeding and controlling drug release suggesting that

such aerogel have potential application in the treatment of both bleeding and chronic wounds.

Respiratory Drug Delivery Systems

The peculiarity of the respiratory system, the nasal tract and the pulmonary biopharmaceutics require careful formulation work taking into account the specific device used to deliver the selected drug. The prime focus here was on powdered nasal formulations and dry powders (DPI), with the aim to overcome solubility, stability and bioavailability issues. The prime focus here was on systems for treat symptoms, reduce complications and improve quality of life of cystic fibrosis patients. Respirable powders have been produced, with or without the use of excipients, containing natural antioxidants, several antibiotics, and NSAID formulated using either mini- or the nano-spray drier [9-10]. The latter concern, due to the lack of specific tests to predict the in vitro dissolution of drugs from pulmonary formulations and the importance of API dissolution in the fluids lining the lower airways, focused to set-up a simulated cystic fibrosis mucus model to evaluate API in vitro dissolution and permeation [11].

Nanomedicines for cancer therapy

Nanomedicine holds great promise for the development of innovative cancer therapies, including targeted therapy, RNA-based approaches, and combined regimens. Despite progress in this field, challenges remain, including high recurrence rates and multi-drug resistance. Conventional anticancer drugs also cause systemic toxicity due to their lack of selectivity. mRNA targeted therapeutic approaches using nanotechnology show a great promise, particularly in combination with chemotherapy. In this broad and appealing field of research we focused on novel polymeric constructs [12] and nanomedicines to overcome these challenges.

Core/shell systems are based on inulin INU [13], which we used as a natural alternative to polyethylene glycol (PEG), offering advantages such as being natural, non-immunogenic, and functionalizable. Nanoparticles with sizes ranging from 50-200 nm, capable of delivering drugs with different hydrophobicity, such as doxorubicin and sorafenib tosylate with unprecedented high loading (EE > 90%), have been obtained [14]. The research is currently focused on developing nanostructures for chemo/siRNA combinations.

Nanoparticles to reach the tumour site, deliver drugs, and overcome barriers. Our approach involves designing libraries of polycationic and amphiphilic copolymers based on inulin and aliphatic polyesters, forming polyplexes, core/shell, or multilayer nanoparticles after interaction with nucleic acids. These INU-based systems are intended to be used to deliver conventional drugs and therapeutic siRNAs simultaneously.

Customizable dosages and constructs by 3D- printing technologies for personalized medicine

The growing attention towards the therapeutic needs of individual patients have been the drive to the investigation of new production methods for customized pharmaceutical forms. Among all, 3D printing has shown great potential as evidenced by the numerous scientific papers published in recent years. The main focus was on how 3D different technologies can be used and specific new printable materials produced and handled safely so that the benefits of personalized drug delivery can be realized.

As to the so-called Fused Deposition Modelling, the research focused on the optimization of the loading of API, both into the filament in the pre-printing and post- printing steps [15]. Semi Solid Extrusion technology enabled the use of excipients widely used in therapeutic in conventional methods production, such as alginate hydrogels. Two different approaches have been envisioned to achieve the goal of printability. Firstly, optimization of a coaxial printing process to obtain an extemporary ionotropic gelation in situ [16] and later, a pre-printing ionotropic gelation (pre-crosslinking) both able to modulate the physico-chemical properties of the alginate matrix for the printing process [17].

Answering needs of the tissue engineering and improving outcomes for implantable surgery, 3D printing is an excellent tool to produce bone scaffolds. With the aim of obtaining new printable pharmaceutical materials, we designed novel PCL-based polymeric blends with advanced properties for bone tissue engineering applications, tailored to Fused Filament Fabrication and Semi Solid Extrusion [18] techniques. The study has allowed to optimize scaffold composition by considering factors such as structural polymer, organic and inorganic additives, and drugs that can aid in osteointegration. Our studies led to optimize 3D printed bone scaffolds in terms of controlled pore architecture, surface texture, and composition. A further advancement has been provided by current design of 3D printed macroporous scaffolds of PCL and inulin-g-P(D,L)LA with more hydrophilic surface character. Such scaffolds provide an optimal environment for bone cell growth and differentiation.

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Antibodies (MISMAs) as artificial counterpart of conventional antibodies in molecular recognition

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Molecular recognition is a desired ability for molecules involved in various technological applications, in particular in the field of life sciences. Molecules with this property, indeed, are able to selectively bind compounds with biological importance mainly for diagnostic, theranostic, pharmacological, drug delivery and research uses. Molecules of this type are present in nature, such as antibodies, which are normally produced by immune cells. However, traditional antibodies present several drawbacks such as limited stability, unsuitable pharmacokinetics, inefficient tissue penetration and impaired interactions with the immune system. Last but not least, conventional antibodies require high production costs and suffer from ethical issues in *in vivo* tests on animals [1].

An interesting alternative consists of synthetic antibodies made from polymers. In this context, Molecular Imprinting represents a very promising and effective technology for the preparation of polymeric matrices characterized by receptor-like properties. Molecularly Imprinted Polymers (MIPs), indeed, are synthesized by polymerizing functional and crosslinking monomers around a target molecule called a template and their selective recognition abilities are due to the formation of a complex between the target analyte and the selected functional monomers during the pre-polymerization step. Being synthetic materials, MIPs are robust, physically and chemically stable in a wide range of conditions including temperature and pH, and more easily available due to their low-cost, reproducibility and relatively fast and easy preparation compared to their biological counterparts. Therefore, this class of materials combines the robustness of polymers with the selectivity of natural receptors finding potential application in the field of antibody mimics.

In the present study, plastic antibodies based on MIPs capable of selectively binding a portion of the novel coronavirus SARS-CoV-2 spike protein were developed [2].

After *in silico* analysis, two different imprinted materials, namely MISMA1 and MISMA2, were synthesized by inverse microemulsion polymerization using acrylamide and a mixture of acrylamide and acrylic acid as functional monomers, respectively. The particle size distribution of MISMAs as evidenced by both DLS and NTA confirmed that the size of both MISMA nanoparticles was around 60-70 nm. The performed binding studies highlighted the capability of both the prepared imprinted polymers to recognize and bind a higher amount of the target molecule compared to the corresponding non-imprinted ones. In order to further confirm the ability of MISMAs to bind the SARS-CoV-2 RDB and, thus, inhibit its interaction with the ACE2 receptor, an inhibitor screening assay kit was used. This kit is characterized by a high sensitivity of detection of the mFc-tagged Spike protein (RBD) by HRP-labeled anti-mouse-Fc. The achieved results confirmed the capability of the developed plastic antibodies to reduce the binding of RBD to its receptor ACE2 in a concentration-dependent manner.

Finally, inhibition of SARS-CoV-2 viral replication by MISMAs was evaluated in Vero cells exposed to a viral isolate purified from a SARS-CoV-2 infected patient. For this purpose, the cells were infected in the absence and presence of MISMAs at concentrations ranging between 10 and 500 ng μL^{-1}. MISMA2 concentration below 100 ng μL^{-1} efficiently inhibits viral growth, with the maximum effect of 99 ± 0.5% of inhibition observed with 20 ng μL^{-1} of the polymeric nanoparticles.

In conclusion, the obtained results confirmed the ability of the synthesized nanoparticles to significantly exert antiviral activity *in vitro*, suggesting their potential use as MIP-based plastic antibodies devoted to blocking the function of the viral spike protein. These results support their use as an effective alternative to the expensive traditional antibodies often unreliable due to their restricted stability.

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Wednesday, September 13
Session 10

Room B - Sala Zodiaco

Chairpersons *Pietro Matricardi – Lucia Zema*

10.50-11.15 Lipid nanoparticles for RNA delivery: potentialities and strategies to foster their clinical use

Giuseppe De Rosa, University of Naples Federico II

11.15-11.40 Liposomal therapeutics: the never end story in drug delivery

Christian Celia, University of Chieti G. d'Annunzio

11.40-12.05 Multifunctional and bioactive polymers for tissue engineering and drug delivery

Giuseppina Sandri, University of Pavia

12.05-12.30 3D printing in pharmaceutics: how to use it?

Luca Casettari, University of Urbino Carlo Bo

12.30-14.30 Lunch

Lipid nanoparticles for RNA delivery: potentialities and strategies to foster their clinical use

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Università degli Studi di Napoli Federico II, Dipartimento di Farmacia

RNA delivery represents one of the key tools for new therapeutic strategies. Messenger RNA (mRNA) in can open new horizons in the treatment of diseases characterized by an altered protein expression. The two approved RNA-based vaccine clearly demonstrated the potentialities of mRNA for vaccination, which can be extended not only to the prevention of viral infections but also in the fight against cancer. On the other hand, from the discovery of RNA interference (RNAi) the knowledge on the pivotal role of non-coding RNA oligonucleotides, e.g. small interfering RNA (siRNA) and micro RNA (miRNA), in regulating cell process boosted research studies for using these synthetic RNA fragment as new potential drugs.

However, the development of RNA-based therapies is hampered by poor biopharmaceutical profile, with rapid degradation in biological fluids and negligible uptake into the cells.

Following the approval of Onpattro® and with the success of the two RNA_based vaccines Comirnaty® and Spikevax®, lipid nanoparticles (LNPs) are considered the leading solution to develop RNA-based therapeutics. Our research group, demonstrated that LNP can be successfully used for the delivery of non-coding RNA, e.g. miRNA, for the treatment of different form of cancer [1,2]. Targeted LNP encapsulating miRNAs have been also proposed [3,4]. LNP encapsulating miRNA have also been used to overcome chemoresistance occurring in the case of immunotherapy [5,6]. Recently, we also demonostrated the ability to use LNP to deliver therapeutic miRNA in the CNS, to treat brain metastasis [7]. Finally, LNP encapsulating miRNAs have also been proposed to reduce the brain damage following ischemia (submitted manuscript).

Despite the success of lipid nanoparticles (LNP), some issues still need to be addressed. Indeed, the poor physical stability of RNA-encapsulating LNP requires low temperatures for storage and transport. Moreover, scale-up of the formulations developed at lab scale remains a challenge. To address these issues, our research group developed a novel nanotechnology approach named lipid self-assembling nanoparticle (SANP) as alternative platform for RNA delivery. Lipid SANP formulations, previously developed by our group for the delivery of bisphosphonates [8,9,10] have shown remarkable biocompatibility, high RNA encapsulation efficiency, and enhanced intracellular release. Furthermore, SANP have been designed to be prepared at room temperature immediately before use by simple mixing of three components, namely, calcium/phosphate dispersion, RNA and cationic liposomes. By using this approach, the RNA can be stored and used in a lyophilized form, which ensures greater stability against degradation compared to freezing. SANPs developed for the delivery miRNA [11] or siRNA [12] in the treatment of different tumors have been proposed. SANPs encapsulating mRNA for vaccination purpose are also under investigation. Further development includes the inclusion of "bioactive" components into the SANP able to prevent neuron damage following oxidative stress, thus making this technology of interest for the treatment of neurodegenerative diseases (*submitted manuscript*).

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LIPOSOMAL THERAPEUTICS: THE NEVER END STORY IN DRUG DELIVERY

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Liposome are widely used colloidal nanocarriers for drug delivery and selective targeting of biomolecules, and they are tailored to deliver therapeutic cargos [1]. Alec D Bangham, a British hematologist at the Babraham Institute in Cambridge described for the first time liposomes, with negative staining, as a model of biological membranes; however he guessed for the first time their potentiality as drug delivery.

The pioneer of liposomes in drug delivery was in 1971, Gregory Gregoriadis who proposed the use of liposomes for drug delivery applications, while the modern era liposomal therapeutics starts in 1990 with AmBisome® and Doxil® approved from Food and Drug Adminstration for clinical injection.

The boost of liposomal technology and the real step for the future comes out with Pfizer and Moderna companies which provide liposomal platform for RNA delivery and efficacious contrast of COVID-19.

In this scenario, our group would like to share with Italian scientific community of pharmaceutical technology, drug delivery and nanomedicine, our expertise, as well as past results and current updates in liposomal therapeutics.

This voyage starts some years ago with gemcitabine liposomal formulations for anticancer therapy [2]. We formulated, gemcitabine chemotherapeutic drug in stealth liposomal nanomedicine and explored molecular mechanisms, biosafety, pharmaceutical, biopharmaceutical and in vitro effects of this nanoformulation in different models of cancer cells [1]. However, we want to demonstrate that liposomes are not only limited to 2D cell culture models but they are safety and efficacious in vivo, and that the systemic injection of liposomes improves the therapeutic effect of cargos and many times increases significantly therapeutic in vivo effects compared to commercial native drugs [3a,b].

By moving in this direction, we demonstrated that liposomal therapeutics can target selectively in vivo specific tissues and had some benefits in pathological animal models thus increasing biopharmaceutical properties and therapeutic effect of cargos with a lower dosage than conventional and marketed drugs [4a], and targeted liposomes allow a dual targeting of pathological models (i.e. breast cancer pro-oncogenic macrophages) both in vitro and in vivo thus blocking physiological promoters of diseases which are present in cellular micro environment as well as as mutated cells or carcinoma [4b].

Our focus and interest want to further explore the potentiality of liposomes and their therapeutic impact when they are combined with physical stimuli, implantable devices and inorganic materials. We applied therapeutic liposomes in tumor pancreatic models in combination with hyperthermia which modify angiogenetic vessels of tumor masses and improve significantly local permeation of drugs. We had demonstrated that the combination of hyperthermia and lipsomal therapeutics decreased significantly the tumor masses and blocked the tumor progression in pancreatic animal models at low dosage of injected drugs [5a].

Hyperthermia was not orphan example how physical elements or inorganic materials can be combined with liposomal therapeutics and have a final synergic effect in therapy. We further loaded liposomal therapeutics in implantable titanium micrometric capsules and control the release of liposomal therapeutics, their confinement and stability inside the implantable microdevice and the therapeutic effect of cargos after its release form liposomes which are previously released from implantable micro devices through silicon nanochannel membranes [5b]. This approach opened the opportunity to combine different devices for therapy and modify the applications of liposomes.

To have a second option for liposomal therapeutics and inorganic materials, we combined porous silicon multistage microdevice and small liposomal chemotherapeutics and tests in vitro in cell culture models [6]. Our approach demonstrated that oxaliplatin effect on colon cancer models increased significantly by combining porous silicon multistage device and liposomal chemotherapeutics and the drug effect was increased by the combination of nano- and micro- carriers through a synergistic approach [6].

Our voyage was continuing and we patented liposomes which can deliver simultaneously RNAi/DNAi and therapeutic cargos, diagnostic agents, molecular molecules and get specific targeting of pathological tissues. The revolution of double bilayer asymmetric vesicles or SVAs was that bilayer structure, which is specific of liposomal therapeutic are not modified in the external layer but internal liposomal layer has lipid structure suitable to suite RNAi/DNAi and make stable complex with the genetic material.

The high versatile of liposomal therapeutics for composition, size, shape and surface properties was a further opportunity in this fantastic voyage. In this attempt, we modified the interface properties of liposomal therapeutics to have super stealth and very long-circulating liposomes which are maintained very stable and for long time in blood circulation without loosing PEG coating [7a] and increase the efficacious in

metastatic breast cancer models both in vitro and in vivo [7b], or post-inserted PEG to modify commercial liposomal products and have a customized and precision nanomedicine for clinical perspectives [7c].

Our voyage is ending now at the final stop at this moment but this is not the end of our story that will continuous and coming soon back for the next never end story.

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MULTIFUNCTIONAL AND BIOACTIVE POLYMERS FOR TISSUE ENGINEERING AND DRUG DELIVERY

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In the present abstract the research lines active at the Department of Drug Sciences of Pavia University and focused on the development of formulations based on multifunctional and bioactive polymers aresummarized. The research on such materials starts in Pavia in the 90s with the study of natural polymers as mucoadhesive agents and penetration enhancers. Some of them, named multifunctional polymers, proved to play an active role in tissue repair processes and, when opportunely derivatized, to act also as stabilizers ofO/W emulsions and nanoemulsions.

In the last years the research has been focused on the employment of such polymers for the development of:

i) **therapeutic platforms intended for tissue repair**;
ii) drug delivery systems aimed to improve bioavailability of drugs with poor solubility/permeability

Development of therapeutic platforms for tissue repair

Mucosal lesions

Mucoadhesive and ion- and/or thermo-sensitive in situ gelling systems have been developed. They were based on a proper combination of κ-carrageenan or methyl cellulose, as multifunctional polymers able to undergo sol-gel transition upon administration**,** cellulose derivatives as mucoadhesive agents and xyloglucan as moisturizing excipient. The occurrence of a synergistic effect among polymers in terms of rheological properties and capability to interact with mucosa was functional to prolong the permanence time of the formulations at the application site and to guarantee a protective action towards ulcerative lesions from additional damages [1, 2]. Moreover nanofibrous scaffolds based on polysaccharides (chitosan (CHS), alginate (ALG) and gums) and proteins (gelatin (GL) and fibroin) have been desiged to be implanted into the periodontal pocket to restore tissue integrity [3].

Skin ulcers

Different formulations (films, nanofibers, sponge-like and particulate systems) based on biopolymers able to restore native functions of the damaged skin were developed. Dressings able to guarantee a combined delivery of PL and an anti-infective model drug, vancomycin hydrochloride (VCM) in the chroniculcers were developed [4]. A simple method was set upfor the preparation of HA core-shell particles, loaded with PL and coated with calcium ALG, embedded in a VCM containing ALG matrix.Subsequently innovative approaches aiming at the development of green and sustainable process were developed and physical crosslinking achieved to avoid chemicals. At this purpose electrospinning was used to prepare nanofibrous membranes entirely based on polysaccharides, starting from aqueous polymer blends [5]. CHS/chondroitin sulfate (CS) scaffold loaded also with Ag nanoparticles, nanocomposites and clays, proved effective in preclinical models in vivo (murine burn/excisional model) and in vitro (fibroblasts and HUVEC) [6-9]. Electrospun nanofibrous scaffolds based on maltodextrin and α-amino acids cross-linked via Maillard-type reaction were designed [10]. This crosslinking conferred to the scaffolds distinctive properties as antioxidant ones due to the presence of melanoidins that possess a strong antioxidant activity. Smart nano-in-microparticles based on polysaccharides (maltodextrin or dextran) and amino acids, and doped with antibacterial nanoparticles (CuO or ZnO NPs) were developed [11]. The resulting 3D structures proved to control tissue moisture and to act as a scaffold to enhance cell migration from the surrounding healthy tissue, cell adhesion and proliferation.

Tendon damages

Recently scaffolds having the ability to mimic the structural, biomechanical and biochemical functions of the extracellular matrix and the native tendons were designed. Electrospinning and freeze-drying were used to manufacture porous and aligned nanofibers and 3D scaffolds. polymers were doped with inorganics to increase the mechanical properties and the biocompatibility, improving cell adhesion, proliferation and differentiation and tissue healing potential.

Electrospun hybrid tubular scaffolds were enriched with hydroxyapatite nanoparticles and extemporaneously loaded into the inner cavity with PL,with the aim of leading to complete post-surgery functional regeneration of the tissue. The scaffold obtained enhances tenocytes and osteoblasts adhesion and proliferation [12].

Nervous tissue injuries

In the last five years, the research group has spent efforts in the design and development of formulation intended for the treatment of spinal cord (SCI) and peripheral nerve (PNI) injuries. Nanofibers and freeze- dried polymeric matrices based on CHS, ALG and gellan gum (GG) have been developed for nerve tissue regeneration [13, 14]. In particular, in recent works, theuse of spermidine (SP), an endogenous polyamine, wasinvestigated in association with ALG or GG for the development of nanogels and nanofibers. SP showed a dual role, acting as cross-linking agent for ALG and

GG and as antioxidant and anti-inflammatory compound having a neuroprotective effect [15, 16].

Development of drug delivery systems aimed at improving drug bioavailability

Among amphiphilic bioactive polymers, some work has been performed in the last few years about hydrophobic derivatives of HA for nanosystem development; as an example, HA hexadecylamine was used for mucosal delivery of the antifungal drug clotrimazole (CLO), whose concentration could be increased about 36-fold compared to the drug inherent aqueous solubility [17]. However, most of the work involved the study of CHS derivatives obtained by the ionic interaction of this polysaccharide with fatty acids. In particular, chitosan oleate (CHS-OA) formed by the interaction of CHS with oleic acid presents a peculiar ability to stabilize O/W emulsions and nanoemulsions (NE) having as oil phase either essential oils or organicsolvents [18].

The stabilization of ethyl-acetate or $CHCl_3$ NEs with CHS-OA allows the one-step preparation of nanoparticles (NPs) based on biodegradable polymers (for example PLGA) coated with a shell of CHS-OA in which CHS is anchored to the PLGA core thanks to oleic acid moieties, while the hydrophilic chains form the shell towards the aqueous environment.

The resulting strong positive charge of the NPs can be exploited for the ionic adsorption of counterions, that in turn modify the NPs surface. This mechanism was exploited for the association of NPs with a fluorescent cyanine (ICG) for photo dynamic therapy (PDT) application. Although the sensitivity of the ionic interaction to medium dilution suggests limiting the use of the ICG NPs for loco-regional delivery, when ICG was associated with NPs the irradiation always produced the statistically significant reduction in Caco-2 cell viability compared with the irradiated ICG solution. This can be explained by easierinternalization of NPs by endocytosis and higher concentrations of ICG associated with NPs [19].

Both in NE and in NPs, the shell maintains some of the properties that make CHS particularly interesting indrug delivery, such as mucoadhesion, positive charge and interaction with cell membranes, absorption promotion, anti-infective and immunomodulatoryactivity.

Some of these effects are supported by the presence of oleic acid, which also participates with anti-infective behavior and absorption promotion properties.

More recently, hydrophobically-modified CHSs have been studied as stabilizers for nanosuspensions, obtained by wet milling, of apremilast, an anti-inflammatory drug PDE4 inhibitor, BCS class IV, authorized for psoriasis treatment.

A nanosuspension sample was obtained by using Lutrol F127, well established as a stabilizer in wet milling, for comparison purposes. Quite differently from what observed in the case of NEs, CHS-OA resulted not suitable to stabilize the apremilast nanosuspension, while nanocrystals stabilized with chitosan association with Na dodecyl sulphate (CS-SDS) or Na cholate (CS-cholate) were obtained with dimensions slightly higher than 400 nm after only 6 hours milling. The ratio between CHS and the two anionic surfactants was studied, to obtain the positive total charge of the nanoparticles. The prevalence of CHS at the NPs surface is in fact relevantfor the mucoadhesion behavior that was confirmed *in vitro*, by the interaction with a mucin solution. After 20days of conservation at 4°C the sample stabilized with CHS-cholate maintained dimensions comparable to the initial ones (from 473±4 nm to 457±70 nm) while for CHS-SDS dimensions increased (from 453±30 nm to 799±11 nm), indicating less efficient stabilization.

Slight shift of the melting temperature of apremilast to lower values is observed for all the nanosuspensions and can be attributed to the intimate mixing of the two components and to the reduction of dimensions to the nanometers range. The decrease in the specific enthalpy of melting of the drug recorded for these systems could also be attributable to a slight loss of crystallinity during the formation of the nanosystem. FTIR and X-ray analysis supported this possible explanation. The dissolution profiles of CHS-cholate and F127 nanosuspensions showed a clear improvement in the dissolution rate with respect to the active and its physical mixtures with the excipients(over 80% in 15 minutes vs less than 40%).

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3D PRINTING IN PHARMACEUTICS: HOW TO USE IT?

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The 3D printing technologies gained great attention in the pharmaceutical field thanks to their flexibility and to the possibility of producing personalized dosage forms and devices.
Taking advantage of the flexibility of additive manufacturing, we explored different approaches to using 3D printing technologies in the pharmaceutical field. Manufacturing processes, dosage forms, medical devices, and analytical systems have been our focus. Among the 3D printing technologies, we investigated the efficient applicability of fused deposition modeling (FDM) and direct powder extrusion (DPE), both techniques relying on the extrusion process.
Compared to conventional manufacturing techniques, the main advantage of the usage of additive manufacturing is that it is possible to do rapid prototyping of almost any model and so, to personalize the final object based on patients' or researchers' needs. Here, as a medical device, we present the development of intravaginal rings with antifungal properties produced with FDM using thermoplastic polymers such as polyurethane and ethyl vinyl acetate (EVA) loaded with clotrimazole, and bifonazole [1]. The rings were efficiently produced after the optimization of the drug-loaded filament and then tested *in vitro* to demonstrate their efficacy. Based on the type of polymer and drug, it was possible to modulate the drug release, which resulted sustained over a week with complete inhibition of *C. Albicans* after 5 days. These rings represent a proof of concept for the treatment of recurrent vulvovaginal candidiasis as acute or preventing treatment.
With the application of the DPE technology, which presents the advantage of producing the final products starting from a mixture of powders or pellets, we demonstrated the processability of polyhydroxybutyrate. This biocompatible and biodegradable excipient was loaded with a model drug (acetaminophen) and then printed in a square geometric form with a prolonged drug release of up to 20 days [2]. DPE technology was also applied to produce transdermal patches made with EVA. Here, we demonstrate the possibility to select different grades of the polymer to be in accordance with the thermal stability of the selected drug to overcome the problem of high-temperature processing during extrusion. The patches were characterized with thermal analysis to evaluate the influence of the printing parameters in the final products.
As manufacturing tools, using FDM, we developed 3D printed microfluidic chips using polypropylene (PP). This polymer secured the best compatibility with most of the solvents, drugs, and excipients in the pharmaceutical field. The developed devices presented two different channel designs that gave an effective passive micromixing to the solvents flowing into them [3]. Taking advantage of these devices, we formulated a wide library of innovative nanocarriers using lipids (*e.g.*, liposomes, solid lipid nanoparticles), polymers (*e.g.*, PLGA), proteins (*e.g.*, keratin), and polysaccharides (*e.g.*, chitosan) in a controllable, tunable, and scalable way [4]. With these devices, we wanted to widen the accessibility to the microfluidic technology that resulted as the most promising tool for the massive production of nanocarriers.
Moreover, with a 3D printed nozzle device we are exploring the solution blow spinning (SBS) technology with the aim to produce drug loaded fibers for biomedical applications.
Finally, with the aim to develop analytical systems that can be used in the pharmaceutical field, we designed and produced a 3D printed vertical diffusion cell (VDC) that can be efficiently used instead of glass ones to evaluate both drug release and permeation [5]. These VDCs made with polypropylene resulted comparable in terms of analytical results with the commercially available glass-based cells being less expensive and less fragile.
3D printing has opened a new era in the pharmaceutical field giving the possibility to develop personalized objects and prototypes in an easier and more accessible way. We strongly believe that in the close future, 3D printing will revolutionize the approaches to producing personalized medicines and pharmaceutical-relevant manufacturing and analytical devices.

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POSTER N. 1

NANODISPERSED ESSENTIAL OILS AS BIODEGRADABLE AND BIOCOMPATIBLE TREATMENTS TO IMPROVE SANITISATION AND FERMENTATION OF THE ORGANIC FRACTION OF MUNICIPAL SOLID WASTE

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The Essential Oils (EO) are natural compounds composed of a mixture of substances produced by plants as a defence mechanism. Due to thewide selection of molecules contained in each EO, theyhave different and multiple properties: antimicrobial, anti-inflammatory, immunomodulatory, anticancer [1], analgesics, expectorants and antitussives and for this reason their application can be in different fields, such as pharmaceutics, food, cosmetics, and agriculture.

However, the EOs are particularly sensitive to exposure to environmental factors such as oxygen, light, pH, humidity reducing or causing the loss of biological activity. Furthermore, they contain some volatile molecules even at room temperature [2]. All these factors make the EOs difficult to use.

In this scenario, nanotechnology has developed strategies through different delivery methods in order to overcome these critical issues for example many researches have been focused on the encapsulation of EOs in Nanoemulsions (NEs), in order to protect the sensitive active components, to facilitate the absorption of EOs in aqueous matrices and to obtain a prolonged and constant release of the active molecules from theoil over time.

NEs are colloidal dispersions composed of oil, surfactant and an aqueous phase, the oil droplets diameter is about 10–200 nm and they formed only by using a high energy method. The chemical composition, the concentration of components and the process condition play an important role for NEs physicochemical properties. In fact, they can undergo different destabilization processes, for example Ostwald ripening, which consists of an increase of droplet size with aging time, mainly in the first 24 h, and it involves a diffusive transfer of the essential oil from smaller to larger droplets, creating an unstable sample.

The aim of our research project is to exploit antimicrobial essential oils activity in order to be nebulized on compost.

Composting is commonly used as an effective practice for waste treatment. It converts organic waste to a humus-like substance with high organic carbon content and biological activity and for this reason not onlyhelps to solve the problem of waste disposal but also improves soil properties and crop productivity. However, the compost microbiological quality plays animportant role because an uncontrolled compost can produce bad smells due to the anaerobic process and it also can be characterized by the presence of numerous pathogenic microorganism that could spread in the surrounding environment causing pathogenicity [3]. Hence the need to make the compost microbiologically safe.

Among EOs, Thyme EO (TEO) has been selected because of its antimicrobial, antiseptic, antiviral, nematocidal and antioxidative properties [4] and for its characteristic odour, to formulate NEs to be nebulized on compost for its 'biostabilization'.

TEO has also a particular behaviour due to its very low viscosity, high volatility, and its appreciable solubility in water. Comparing TEO to other EOs, this particular feature makes more difficult to vehicle TEO in a NE and it makes the NEs more susceptible to Ostwald Ripening [5]. In this work we also studied ways to reduce this phenomenon [6].

Different ternary phase diagrams have been constructed in order to identify the best combinations between oil, surfactant and water to formulate a stable NEs with suitable characteristics with lower percentage of surfactant. Different strategies have been adopted,for example changing surfactants and mixing TEO with another oil in order to obtain stable NEs.

The selected formulations were analysed in terms of hydrodynamic diameter, ζ-potential and polydispersion index (PDI). Stability studies were carried out at different temperature conditions (55°C and 70°C) simulating the composting chamber ones. Furthermore, NEs integrity was tested after the nebulization process. NEs showed good stability at two different storage temperatures (25°C and 4°C) for three months and after nebulization. Release studies of OE components at 25°C and 60°C and of Nile Red, a lipophilic probe, at 25°C and 60°C were performed.

Finally, with the aim to study how the different formulations can influence the release of EO volatile fraction from a qualitative and quantitative point ofview, the samples were subjected to analysis by Solid Phase Microextraction-Gas Chromatography-Mass Spectrometry (SPME-GC-MS) techniques.

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POSTER N. 2

TACKLING BIOLOGICAL BARRIERS TO ANTIGEN DELIVERY BY NANOTECHNOLOGICAL VACCINES (NANOTECHVAX)

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The project aimed to set up a next generation of nanovaccines through a deeper understanding of the impact of the delivery systems on the immune response. Hence, the interactions between different nanocarriers (NCs) and the immune system was studied to improve safety and efficacy of vaccine delivery through the design of appropriate nanoparticulate carriers. The project was performed by a close cooperation between six different Research Units (RUs) converging to the common aim. To reach this goal, the five RUs designed a panel of antigen-loaded NCs, with different composition and physico-chemical properties. The type of delivery systems spanned from polymeric nanoparticles to liposomes, from nanogels to microneedles, reflecting the expertise of the RUs involved. The most suitable administration route (parenteral, mucosal, topical, pulmonary) was identified for each NC that was tested accordingly using *in vitro/in vivo* models. Ovalbumin (OVA) was employed as a model antigen, and all formulations were subjected to physico-chemical and technological characterization (size distribution, ζ-potential, EE%, stability). Finally, immunological evaluation was performed by the RU-CNR-NA to gain information on the efficacy of antigen presentation and on the safety as to induction of inflammation by NCs.

RU-UniCT focused on the design and development of nanogel (nGEL), a suitable vehicle for non-invasive vaccine delivery able to elicit both systemic and mucosal immunity. The composition of nGEL was studied according to Quality by Design approach, taking into account the polymers concentration, type and ratio and also critical parameters such as ionic strength, temperature and pH. nGEL based on dextran sulfate and ε-poly-lysine were designed by Response Surface Methodology, loaded with OVA and/or OVA-FITC and subjected to physico-chemical and technological characterization, including *in vitro* release, mucoadhesive properties and immunological activity. Finally, *in vivo* studies were performed on mice by an X-ray/optical imaging system to evaluate the potential of nGEL for mucosal vaccination. Results demonstrated a prolonged localization of OVA-FITC loaded-nGEL in the nasal mucosa compared to the free protein after nasal administration.

RU-UniCZ developed (hydro)gels made of biocompatible polymers and loaded with NCs prepared by other RUs to obtain multistage depot formulations. Among the various available biomaterials poloxamine 908 (P908), a highly hydrophilic copolymer characterized by reversible gelation at body temperature, was used to obtain in situ-forming injectable hydrogels. The amount of P908 affects the micro-rheology, steady-state, viscosity, and mechanical properties of the systems: in detail, P908 aqueous solutions above 25% w/w of copolymer were characterized by different $T_{sol\text{-}gel}$, a useful shear-thinning behaviour, and a significant physical stability. The evaluation of the release profile of ovalbumin showed a prolonged leakage of the protein, phenomenon that could enhance the immune-response.

RU-UniPR investigated the effect of dissolving microneedles (MN) of different length, prepared by RU-UniCA, on the permeation of model polysaccharide antigens (high m.w. dextrans) across the buccal mucosa. Fluorescent dextrans with 70 kDa and 150 kDa molecular weight, used as model molecules for polysaccharide antigens, were incorporated in dissolvable PVP microneedles of different length (150, 500 and 800 μm). Permeation experiments carried out across pig esophageal mucosa revealed that only 500 and 800 μm-long MN can cross the epithelial layer, allowing for a rapid permeation of dextrans. Microscopy images of the mucosa confirmed that 500 and 800 μm MN were able to penetrate the mucosa, whereas 150 μm MN were not found in the tissue. Results suggest the feasibility of administering high molecular weight molecules across the mucosa using MN arrays of suitable length.

RU-UniNA developed inhalable nanoparticles (iNPs) composed of poly(lactic-co-glycolic) acid (PLGA) for pulmonary vaccination. iNP were co-loaded with OVA and a single-stranded microbial DNA fragment with unmethylated deoxycytidylyldeoxyguanosine dinucleotide (CpG) as vaccine adjuvant. Their surface was engineered with different moieties to achieve muco-inert particles and improve the immune response. All the iNPs were homogeneously dispersed (size <250 nm), had high entrapment efficiency (around 70% OVA and 95% CpG) and displayed negative/positive surface charge depending on composition. Formulations showing the most promising

technological features underwent *in vitro* uptake studies, antigen presentation tests and evaluation of their pro-inflammatory potential in cooperation with RU-CNR-IBBC-NA. Results highlight the potential of iNPs to deliver antigens to the respiratory mucosa.

The RU-UniCA prepared OVA nanoaggregates and vesicular formulations. Nanoaggregates were formed exploiting the nanoprecipitation of OVA in a microfluidic mixer in the presence of high alcohol concentration. Homogenously dispersed particles with high protein loading were obtained and characterized in terms of composition and morphology. Investigations by RU-CNR-IBBC-NA showed that nanoaggregates enhanced the antigen-specific immune response *in vitro* and *in vivo* compared to the free protein with or without alum, used as a clinically relevant adjuvant.
DOTAP liposomes were also prepared and tested for potential nasal administration, while liposomes with different additives (glycerol, hyaluronan, deoxycholate) improved OVA delivery to the skin. In addition, the RU-UniCA prepared and studied the permeation across the skin of polymeric microneedles using low m.w. model drugs.

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A. M. Fadda, C. Sinico, M. Schlich, L. Casula, M. C. Cardia, F. Lai, M. Manconi, M. L. Manca, M. Aroffu (RU-UniCA), P. Italiani, L. D'Apice, A. Corteggio, T. Heinzl (RU-CNR-IBBC-NA), R. Pignatello, C. Carbone, T. Musumeci, A. Bonaccorso, B. Tomasello (RU-UniCT), A. Gagliardi, S. Voci, N. Ambrosio, M. Fresta, D. Cosco (RU-UniCZ), P. Santi, A. Fantini, S. Nicoli, C. Padula, S. Pescina (RU-UniPR), G. Costabile, G. Conte, C. Conte, A. Miro, F. Quaglia, F. Ungaro (RU-UniNA) contributed to this work.

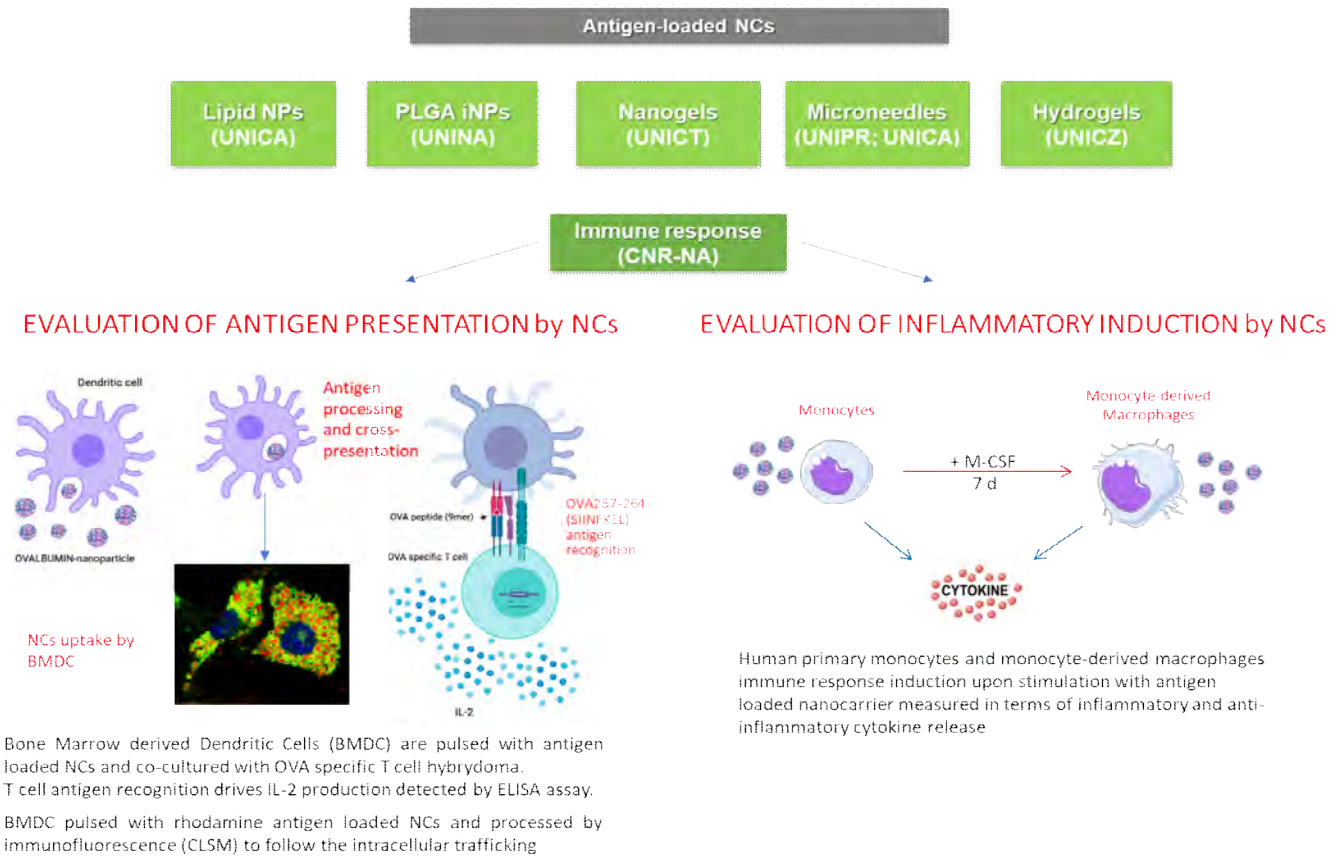

Figure 1. Schematic representation of the research work carried out by the six Research Units to evaluate the impact of the delivery systems on the immune response.

POSTER N. 3

ROS-SENSITIVE MATERIALS BASED ON POLYLACTIC CO-GLYCOLIC ACID FOR CANCER

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Colon cancer is the most frequently diagnosed cancer among the various cancers that normally affect humans on a global scale [1]. Chemotherapy is still mostly administered parenterally, which is a negative factor in terms of patient comfort; therefore, oral administration would be desirable especially for chronic patients requiring long-term treatment [2,3].
From this point of view, polymeric nanoparticles (NPs) are promising for the development of an oral formulation for colon cancer as they protect the drug from various destructive effects of GIT and allow higher local concentrations to be delivered into the colon [4].
The aim of this work was to design, realize and evaluate the performance of polimeric nanoparticles based on PLGA alone or PLGA esterifiedwith 2,2'-[propane-2,2-diylbis (thio)] diacetic acid, loaded with docetaxel (DCX) and docosahexaenoic acid (DHA), as innovative site-specific therapeutic carriers. The technique used to realize nanoparticles was the nanoprecipitation illustrated in the Figure 1.

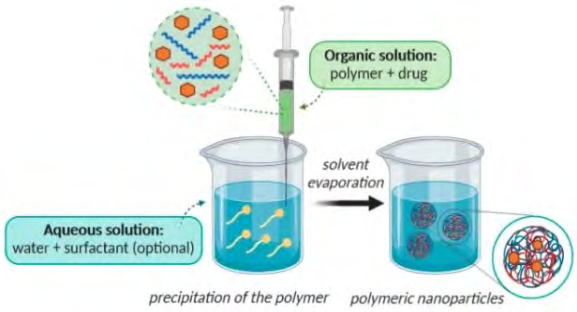

FIGURE 1. Nanoprecipitation technique.

The obtained materials were characterized by FT-IR and ^{1}H-NMR, while dimensional analysis of the nanoparticles obtained was performed by Dynamic Light Scattering and encapsulation efficiency was evaluated. *In vitro* skin permeation tests and antitumor activity on HCT116 cell lines were also conducted. The obtained results showed that the encapsulated drugs are released from the NPs in percentages ranging from 30/35% to 80%, for PLGA-based NPs containing docetaxel and both docetaxel and DHA (Figure 2).

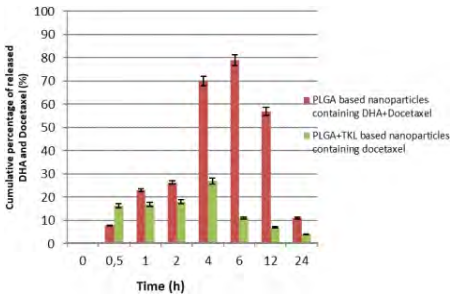

Figure 3. Graph of cumulative release of DHA and docetaxel.

In addition, testing for antitumour activity showed a significant inhibition of cell viability both when the drug was administered alone and in the presence of DHA. The use of this nanocarriers could facilitate the stable and efficient delivery of DCX and DHA through the upper segments of the gastrointestinal tract to the colon. In addition, the presence of the ROS-sensitive 2,2'-[propane-2,2-diylbis (thio)] diacetic acid in their matrix should promote the site-specific release of DCX and DHA in the cancer pathology zone, where high levels of reactive oxygen species could be found.

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POSTER N. 4

ENGINEERED NANOPARTICLES FOR LOCAL DELIVERY OF ANTIMICROBIAL PEPTIDES IN PSEUDOMONAS AERUGINOSA LUNG INFECTIONS

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Antimicrobial peptides (AMPs) are considered one of the rare alternatives to traditional antibiotics in the treatment of multi-drug resistant P. aeruginosa lung infections. Nevertheless, their activity is significantly reduced by their interactions with airway barriers. Along these lines, the aim of this research line was the design and development of engineered nanoparticles (NPs) for the sustained delivery in the lung of two different AMPs, Esculentin 1-21 (Esc 1-21) [1] and M33 [2]. To this purpose, NPs made of poly(lactide-co-glycolide) (PLGA), with a core-shell structure, were engineered by using hydrophilic polymers, such as poly(vinyl alcohol) (PVA) and polyethylene glycol (PEG) in order to modulate surface properties and, in so doing, to improve NP transport through barriers in lung infections (i.e. mucus layer and bacterial biofilm). PLGA-based NPs, encapsulating the AMP were prepared by a modified emulsion/solvent diffusion technique [3-5] (Figure 1).

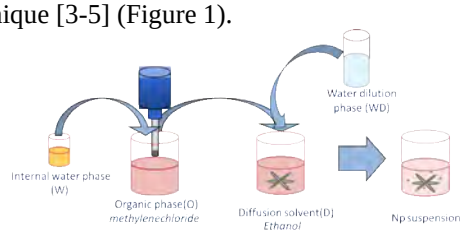

Figure 1: Schematic representation of NP production.

NPs were engineered by using hydrophilic polymer, PVA or PEG, to tune surface properties and to improve NP transport through barriers in infected lungs. NPs were prepared in different formulation conditions, which are reported in Table 1.

Table 1: AMP-loaded NPs formulation conditions.

AMP loaded	Organic phase composition		Water Dilution phase composition
	Component	Amount (mg/mL)	

aqueous layer thickness (FALT). The AMP-loaded NP diffusion through the artificial mucus and bacterial extracellular matrix was evaluated by a transport model based on Transwell® multiwell plates previously developed [3;5]. Finally, the efficacy in inhibiting P. aeruginosa growth *in vitro* and *in vivo* in mouse model of lung infection and the *in vitro* antibiofilm activity were assessed.

Independently upon the adopted formulation conditions, developed NPs showed a hydrodynamic diameter suitable for pulmonary delivery and high AMP entrapment efficiency (more than 80 %). High differences in size and ζ potential were observed changing the hydrophilic polymers. In particular, PEG NPs showed lower size and ζ potential, probably thanks to the stabilizing effect of the hydrophilic shell on particle surface. In fact, the PEG shell appearedthicker than the PVA shell layer (2.18 nm, 3.21 nm and 4.07 nm for PVA, PEG 2000 and PEG 5000 shell respectively).

Table 2. Properties of AMP-loaded NPs.

Formulation	Size (μm ± SD)	PI (mean ± SD)	ζ potential (mV ± SD)	E.E. (% ± SD)
Esc 1-21_PVA	261.0 ± 1.3	0.04 ± 0.02	-7.57 ± 0.23	83.0 ± 2.0
M33_PEG 2000	189.9 ± 28.7	0.07± 0.05	-27.3± 4.9	98.8 ± 0.6
M33_PEG 5000	163.8± 0.4	0.07± 0.04	-24.4 ± 9.4	88.9 ± 3.0

The developed NPs provided a sustained release of the peptides up to a week. Differences in release rate due to the AMP structure and particle properties were observed. Esc 1-21 release appeared faster if compared to M33. The ability of engineered NPs to assist the diffusion of AMP across the mucus layer and the antimicrobial biofilm was confirmed by the transport assay (Figure 2).

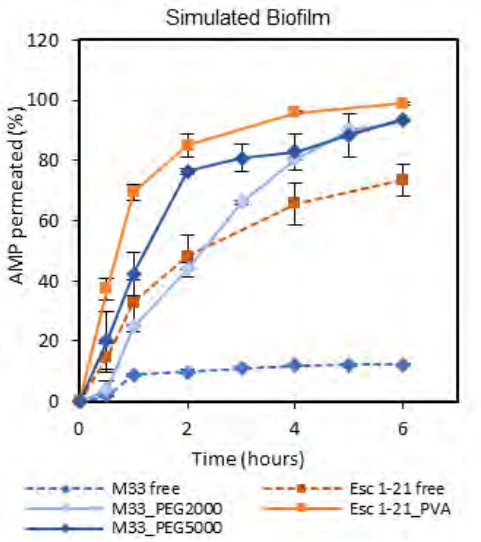

Figure 2: *In vitro* transport of AMP through artificial mucus and bacterial alginates (simulated biofilm), as determined by the Transwell® multiplate assay. Results are presented as the percentage of AMP labelled with rhodamine permeating across the artificial mucus or simulated biofilm in the time.

Furthermore, the unmodified NP size in presence of mucin or bacterial alginates (data not shown) confirmed that NPs are inert, suggesting the ability to easily diffuse across both the mucus layer and simulated bacterial biofilm.
Of note, the encapsulation of AMP into PLGA nanoparticles leads to a preserved the in vitro antibiofilm activity and, in the case of Esc 1-21, a prolonged efficacy in inhibiting P. aeruginosa growth as compared to the free peptides.

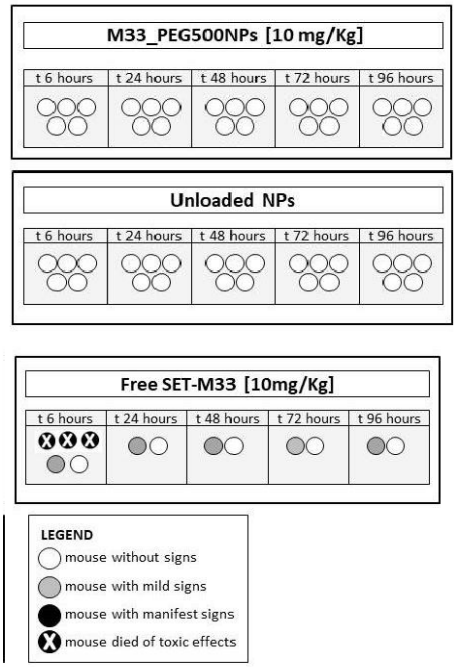

Figure 3. Acute toxicity of M33_PEG5000 NPs, free M33 and unloaded NPs *in vivo*: Mice (represented as circles) underwent i.t. inoculation with 10 mg/kg in a single dose and were monitored for 96 h. Different scales of grey and the X symbols in the circles indicate severity of signs and death, as described in the internal legend.

More importantly, a single intra-tracheal administration of peptide-loaded NPs in a mouse model of P. aeruginosa lung infection resulted in a reduction in AMP toxicity, as in the case of M33 (Figure 3), and a 3-log reduction of pulmonary bacterial burden up to 36 h, in the case of Esc 1-21 (Figure 4), with respect to the control peptide aqueous solution.

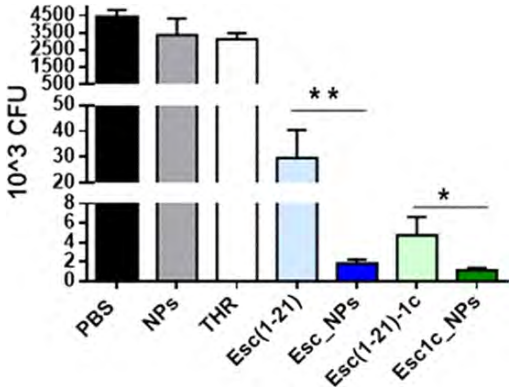

Figure 4. Comparison among unloaded NPs, THR, Esc peptides, and peptides-loaded NPs (final peptide concentration, 0.1 mg/kg) on the CFU numbers of P. aeruginosa in mouse lungs (BAL+ lung tissue homogenate) at 36 h after lung infection**.**

PLGA-based NPs for the delivery of AMPs to the lung with optimized properties were successfully developed. NP surface engineering with hydrophilic polymers can inhibit the NPs interaction with the mucin and bacteria biofilm, leading to inert carriers, which can easily diffuse across the barriers imposed by the infected lung.

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POSTER N. 5

PREPARATION AND CHARACTERIZATION OF MOLECULARLY IMPRINTED POLYMERS (MIPs) FOR GEFITINIB RECOGNITION AND CONTROLLED RELEASE

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Molecular Imprinting represents a very promising and attractive technology for the preparation of polymeric materials (Molecularly Imprinted Polymers, MIPs) characterized by specific recognition abilities for a desired target molecule, called template [1]. MIPs are synthesized by polymerizing suitable functional and crosslinking monomers around the chosen template, to obtain a highly crosslinked three-dimensional matrix, and can find different applications including as sensors, stationary phase in chromatography, solid phase in SPE and artificial antibodies and in drug delivery field. MIPs, indeed, are able to release a therapeutic agent in a controlled manner due to the presence of complementary binding cavities into the polymeric matrix.

In this context, the aim of the present research study was the synthesis of Molecularly Imprinted Polymers for the controlled delivery of Gefitinib (GEF), which is a first-generation epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI) widely employed in the treatment of many cancer diseases including non-small-cell lung carcinoma (NSCLC) [2]. GEF is characterized by a low solubility in aqueous media and a poor bioavailability and, thus, requires a high dose to be effective resulting in the onset of severe adverse effects. In addition, an acquired resistance is observed in patients with NSCLC after one-year treatment [3].

MIPs were synthesized by precipitation polymerization according to the non-covalent approach using methacrylic acid (MAA) as functional monomer and including fluorescein O-methacrylate (FM) in the reaction mixture to provide fluorescent properties to the material, enabling its use as diagnostic agents or for cancer staging. Ethylene glycol dimethacrylate (EGDMA) and trimethylolpropane trimethacrylate (TRIM) were both investigated as crosslinkers and different GEF/functional monomer/crosslinker molar ratios were screened in order to obtain the polymeric materials characterized by the best imprinting efficiency. Non-imprinted polymers (NIPs) were also prepared in the absence of template during the polymerization process.

The synthesized polymeric particles showed nanometric size and fluorescent properties ascribable to the presence of the fluorescein-modified monomer. Furthermore, hydrophilic properties were investigated by protein adsorption measurement, using bovineserum albumin (BSA) as model protein, and water regain experiments. In order to evaluate the imprinting efficacy and selectivity of the prepared MIPs, binding experiments at different times and concentrations were carried out in the presence of standard solutions ofGEF and its analogue Vandetanib (VAN). These studies confirmed the imprinting efficiency and selectivity of all the synthesized MIPs and the bestresults were observed for the imprinted nanoparticles prepared using EGDMA as crosslinking agent and with a GEF/MAA/EGDMA molar ratio equal to 0.5:4:10. Moreover, the performed *in vitro* release studieshighlighted a faster GEF release profile from NIPs,which are characterized by weak and non-specific interactions with the template resulting in a massive release in the short period of time. On the contrary, MIPs allowed a more controlled and sustained release due to the presence of specific binding holes within the polymeric matrices, which are able to strongly interact with GEF molecules. Finally, *in vitro* cytotoxicity studies confirmed the biocompatibility of the prepared polymers.

In conclusion, the synthesized MIPs combined fluorescence properties with highly selective recognition and sustained release abilities, representing a promising material for the development of a novel theranostic platform with a potential application in cancer therapy to improve the therapeutic efficacy of GEF and reducing at the same time the adverse reactions.

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POSTER N. 6

COMBINATION OF ATR-FTIR SPECTROSCOPY AND TAPE STRIPPING TECHNIQUE TO INVESTIGATE THE SKIN DISTRIBUTION OF ETHILHEXYL TRIAZONE ENCAPSULATED IN SPOROPOLLENIN MICROCAPSULES

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The evaluation of the permeation of bioactive substances through the skin plays a crucial role in various fields, such as pharmaceutical, cosmetical, and dermatological ones. Indeed, determining the depth of penetration of substances applied on the skin is an important tool both for therapeutic reasons and for assessing the risk of exposure to certain substances. Skin penetration of active agents is generally evaluated by classical techniques such as tape stripping or cryosectioning, followed by extraction procedures and quantitative analysis by UV/Vis spectroscopy or fluorescence.

However, there are analytical difficulties when the research interest is aimed at determining thepenetration depth of other ingredients of theformulation or when it is necessary to understand whether some innovative drug delivery systems, suchas micro- or nano-structured systems, are capable ornot of penetrating even only partially into the more superficial layers of the skin.

In recent years, ATR-FTIR analysis has emerged as a powerful technique for investigating the permeation of substances in skin models. Combined ATR-FTIR spectroscopy and tape-stripping procedure could be useful to characterize the structural organization of the different layers of the skin and investigate the distribution of substances in its layers, particularly in the SC [1,2]. Moreover, the interaction of the delivery systems with the skin can be investigated by observing changes in the characteristics bands, when the spectra are compared with reference samples.

Recently, an innovative sunscreen delivery system (ETZ-SPMs) composed of ethylhexyl triazone (ETZ, Uvinul® T150) encapsulated in the hydrophobic cavity of sporopollenin microcapsules (SPMs), extracted from *Lycopodium Clavatum* pollen grains, has beendeveloped [3].

Among the so called coral safe-sunscreens, ETZ is a photostable organic UVB filter approved for cosmetic use in Europe in a concentration up to 5%, but possesses some formulative concerns since it is difficult to formulate in water-based products,preferred by users with respect to oily formulations. SPMs were demonstrated to be effective as delivery systems for ETZ, also conferring UVA protection at the system at the sunscreen concentration of use in the cosmetic field. Therefore, the possibility of functioningboth as a release system and as an active ingredient provides these products with added value, together with easy availability and good reproducibility in the production phase.

In addition to optimal protection, the substantivity of the product, i.e. the ability to bind to the stratum corneum and resist simple washing removal, is crucial for a sunscreen to maintain long-term protectionagainst UV radiation under real-life conditions.

Therefore, in order to understand the interactionbetween the formulation and the skin and to determine the amount of sunscreen penetrated in the first layer of the stratum corneum, *in vitro* distribution studies were carried out using porcine ear skin, a widely recognised suitable model for human skin.

The distribution of ETZ in the different depths of the stratum corneum after tape-stripping was analytically determined by HPLC after extraction from the tape-strips and the skin residuals. Furthermore, the obtained results were compared with the ones obtained after applying ATR-FTIR spectroscopy on the stratum corneum tape-strips deriving from the penetration study. Untreated skin samples were used as control and multivariate data analysis was performed to differentiate the spectral data of the skin treated with ETZ-SPMs from the skin itself in terms ofcharacteristic bands of the sunscreen and SPMs.

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POSTER N. 7

DEVELOPMENT OF ODT's CONTAINING METOCLOPRAMIDE USING BINDER JETTING 3D PRINTING

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3D printing is an innovative technology with potential use in personalised medicine. This medical model aims to provide "*the right therapeutic strategy for the right person at the right time"* [1]. It takes into consideration the physiology, drug response and genetic profile of the individual.

The therapy with metoclopramide could be used as a simple model of personalised medicine, this API is used for the prevention or treatment of nausea, vomiting, and gastrointestinal motility disorders. The European Medicine Agency (EMA) recommends doses from 0.1 to 0.15 mg/kg body weight, repeated up to three times daily by oral route [2]. This means that the dose has to be adjusted, particularly, in pediatric patients who also may have problems swallowing a conventional tablet, especially the younger ones. For this reason, orally disintegrating tablets (ODT) could be a valid alternative for the administration of metoclopramide for this category, which requires personalised doses according to their weight and also a dosage form suitable for them.

Binder Jetting methodology (BJ) is one of the most suitable 3D printing technologies for the preparation of ODTs, thanks to its characteristic of producing objects with high porosity [3]. As for direct compression, the powder blends used for the preparation of ODT by binder jetting 3D printing must have suitable flow characteristics and good cohesive properties, moreover, the dosage forms prepared with these materials must be able to disintegrate quickly once in contact with saliva. In previous work, our research group demonstrated the usefulness of co-processed materials for the preparation of ODT prototypes using BJ technology [4].

This work aimed to develop ODT formulations by binder jetting 3D printing containing three doses of Metoclopramide as model API. Blends of co-processed excipients, commonly used for ODT preparation by direct compression have been used. The influence of some process parameters and the physical characteristics of the powder blends on the mechanical properties of the prototypes printed, as well as the API loading efficiency in the dosage forms were evaluated.

The previous results showed that the co-processed approach is useful to prepare solid dosage forms with this technology; however, additional excipients were needed to improve the physical properties of the prototypes. In this work, three different ODT formulations containing 2.5 mg, 5.0 mg and 10 mg doses respectively of metoclopramide were printed.

The results showed that the addition of metoclopramide to the powder blend did not produce notable differences in the mean weight or in the mechanical strength (hardness and friability) or the disintegration time of the dosage forms concerning the characteristic of the prototypes (only excipients) previously evaluated (Table 1). The analytical assay showed that the three formulations had API content between 90% and 110% as required by the compendial test.

Table I. ODTs characterization

ODT Formulation	Weight (mg)		Tensile Strength (g)		Friability	DT*
	mean	SD	mean	SD	(%)	(s)
without MET	123.1	6.2	2422	479	0.5	36.0
MET 2,5	125.4	0.1	2184	149	1.3	49.0
MET 5,0	128.6	0.4	2346	102	1.1	43.5
MET 10	125.9	1.3	2532	157	0.6	98.3

*DT: disintegration time

The BJ technique has been demonstrated to be a suitable technology for the preparation of ODTs

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POSTER N. 8

Design,characterization and optimization of nanostructured vectors for the delivery of Mi- or Si-RNA-Development and optimization of intelligent vectors for RNA delivery.

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Immune-mediated diseases (IMDs) are pathologies characterized by an altered response of the immune system, which leads to consequences such as chronic inflammation of the tissues, pain and a reduction of the quality of life of patients. Nowadays, there are no effective treatments for this group of diseases.

Development of new effective drugs for the treatment of IMDs could lead to an important improvement of the quality of life of patients. Gene therapy may represent the most important strategy for IMDs treatments; recent studies have highlighted the potential of using mRNA based therapy [1].

In particular, a new frontier for the treatment of IMDs could be reached using Gene knockdown therapies with RNA interference (RNAi).

MiRNA are noncoding RNAs composed by double-stranded stem-loop RNA structures with a length of 21–25 nucleotides and dimensions of 13–15 kDa, involved in the RNA interference pathway. This may theoretically be used to target inflammatory mediators. However, miRNA is unstable and susceptible todegradations by exon- and endonucleases, and its negative charges are electrostatically repulsed by the anionic cell membranes; therefore, it is necessary to develop a formulation that allows miRNA to be encapsulated and transported to the site of inflammation.

Lipid nanocarriers (LNCs) represent a non-viral leading vector for mi- or si-RNA delivery [2]; they are usually composed of four kinds of lipids serving to encapsulate Mi- or Si-RNA: an ionizable (or ionized) lipid, a phospholipid, cholesterol, and a polyethylene glycol (PEG)-lipid.

The nanostructure of LNCs has a positive charge, which allows mi-RNA to enter, and contains an aqueous core entrapped by one or more phospholipid's bilayers.

This research work aims to develop a parenteral formulation composed of lipid nanocarriers (LNPCs) containing miRNA [3].

The first step of this work involves the screening and selection of the components necessary for the formulation of the LNCs [4], followed by the production of LNCs.

Material and Method: cholesterol, DMG-PEG-2000, PE 18:0 PEG-2000 were from Merck (Italy)

LNCs with different lipids and composition were produced by the following method. A chloroform lipids solution was evaporated with nitrogen flow in order to create a film, then was kept for 1 hour in a vacuum stove at 37°C. 3 ml of aqueous buffer was added to the samples. After vortexing, the film was left in the refrigerator overnight to allow for proper hydration. Following this, the samples underwent three additional vortex cycles and five freeze and thaw cycles. All samples were then sonicated to further break down any remaining particles or aggregates. Finally, half of the samples were extruded to create a more uniform and consistent film

In particular, three different LNCs were produced: without PEG-lipid, with DMG-PEG-2000 and with PE 18:0 PEG-2000 and were compared by verifying size, Z potential and stability. Results suggest that the formulation containing PE 18:0 PEG 2000 is the best, mainly in terms of stability.

Next steps will involve the use of DOPE-PEG lipid and the verification of the encapsulation efficacy of this LNCs with the use of Poly(A), Poly(U) and, finally, RNA.

Dyinamic Light Scattering (Zetasizer Pro Red Label) have been will be used in order to evaluate the particle size, zeta Z potential (Zpot) and stability of LNCs.

LNCs that will provide the best results will be used to encapsulate mi-RNA.

Chemical-physical properties, stability and encapsulation capacity of the mi-RNA will be characterized with HPLC-DAD-MS, Light scattering, DSC and Cryo-TEM techniques.

The ability of LNCs to transfer mi-RNA to cells will be tested on cell cultures. The last step will be focused on the selection of the components for development of a parenteral formulation.

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POSTER N. 9

ENCAPSULATION AND CHARACTERIZATION OF CIS-PLATIN IN LIPOSOMES FOR LUNG CANCER TREATMENT

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Cis-diamminedichloroplatinum (II) (CDDP) is a chemotherapeutic drug clinically exploited for the treatment of bladder, lung, ovarian, testicular cancer, and solid tumors of head and neck. It is a compound with antiproliferative activity and it is able to interact with DNA and cause cellular damage leading to cell death [1]. Nevertheless, CDDP comes with serious limitations: the main disadvantage of using it as a chemotherapeutic agent is its highly toxic nature, as systemic administration induces serious side effects. Among these are nephrotoxicity, neurotoxicity, and teratogenicity. Moreover, cancer cells may develop resistance to CDDP by increasing the repair phenomena of DNA and increasing tolerance to the drug [2]. Researchers aim to develop new strategies to overcome these obstacles, and nanomedicine is an approach to achieve this purpose [3].

On these bases, CDDP-encapsulating liposomes (Lipo-CDDP) were prepared by *ethanol injection* and *extrusion* methods. They were characterized by size, Z potential, and encapsulation efficiency (EE%) analysis. Lipo-CDDP showed a diameter of around 180 nm, a Z potential of -10 mV, and an EE% of 30%. The drug release profile of Lipo-CDDP was evaluated in RPMI-1640 medium at 37 °C, and we found that 50% of CDDP was released after 48 h demonstrating the controlled release of the drug.

Furthermore, Lipo-CDDP were decorated with conjugates composed of hyaluronic acid (HA) oligosaccharides and phospholipids (Lipo-HA-CDDP) synthesized by M.E. Cano *et al.* [4] with the aim of targeting lung tumoral cells, which overexpress the hyaluronic acid receptor CD44. HA-oligosaccharides-phospholipid conjugates were added directly to the lipid film due to their lipophilic nature, and then Lipo-HA-CDDP were prepared as Lipo-CDDP described above. Lipo-HA-CDDP were characterized for their size (around 230 nm), Z potential (around -30 mV), and EE%, which was the same as that of Lipo-CDDP. Both formulations were assessed for their *in vitro* cytotoxicity and uptake by lung cancer cell lines (A549, H1650 and Calu-3), exploiting fluorescent markers to label liposomes. Results showed higher uptake and cytotoxicity for Lipo-HA-CDDP than for free CDDP and Lipo-CDDP.

Lipo-CDDP and Lipo-HA-CDDP were tested *in vivo*, showing an improved therapeutic efficiency compared to plain liposomes and the free drug.

In conclusion, this study demonstrates that liposomes are useful nanosystems for improving the solubility, delivery, and stability of CDDP. Our preliminary results are promising and will be further investigated by additional *in vitro* and *in vivo* studies, with the purpose of improving CDDP anticancer activity, decreasing side effects, and overcoming the resistance against it.

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POSTER N. 10

CISPLATIN/HYALURONAN COMPLEX: A NOVEL LOCO-REGIONAL TREATMENT IN TUMORS OVER-EXPRESSING CD44 RECEPTOR

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Purpose

In 2022, there will be an estimated 1.9 million new cancer cases diagnosed and 609,360 cancer deaths only in the United States [1]. Tumor specific delivery of chemotherapeutics with less systemic side effects, is an ongoing challenge of chemotherapy. In this context, we proposed a cisplatin (cisPt)-loaded sodium hyaluronate (NaHA) gel for intratumoral therapy. Therefore, this study addresses the idea to deliver cisPt/NaHA complex [2] locally in different cancer, where CD44 receptor is over expressed. Hyaluronic acid is the primary CD44 binding molecule and in many studies has proved a significant ally in developing drug delivery system that demonstrate preferential tumor accumulation and increased cell uptake [3]. To this aim, we investigated *in vitro* the anticancer efficacy of cisplatin/hyaluronan complex *vs.* cisPt alone and the possible enhancement by valproic acid (VA) using 2D and 3D cell models of various cancer cell lines overexpressing CD44. Also, effect of drug exposure time on cell viability of various cancer cell lines was evaluated.

Methods

The anti-proliferative effects of cisplatin/hyaluronan complex and cisplatin with valproic acid was determined in human lung epithelial cell (A459), human pancreatic cancer cell lines (MiaPaCa-2 and $BxPC_3$) and human melanoma cell line (A375 and vemurafenib-resistant melanoma cells (AV)) by MTT cytotoxicity assay after 48h. Effect of drug exposure time on cell viability was assessed. The CisPt/NaHA complex was formulated in NaCl saline (0.9% w/v) using hyaluronan at low (LMW) and high (HMW) molecular weight, namely 10 kDa and 1.330 kDa. Complex formation was assessed by HPLC [2]. Growth and morphology of CisPt/NaHA complex and complex with valproic acid was evaluated also in 3D multicellular melanoma tumor spheroids.

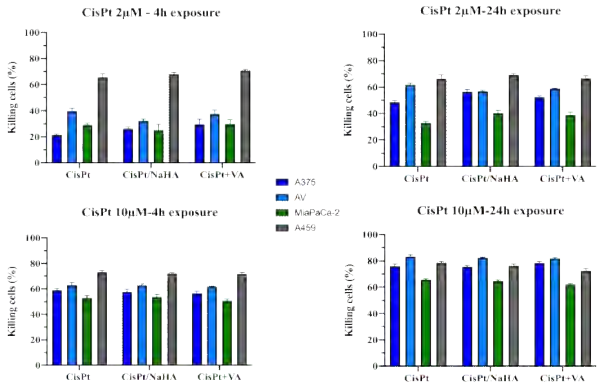
Results

Cisplatin, cisPt/NaHA complex (high molecular weight of NaHA) and cisPt with valproic acid exhibited dose-dependent inhibition in viability of all cancer cells tested (IC_{50} values reported in Table 1).
There was a significant difference in cisplatin-NaHA induced cell killing in cancer cell line. In this in vitro model, the complex with HMW NaHA was as effective as cisPt alone at 48h. The same result was observed by shortening treatment to 2 or 4 hours. In the concentrations used, 76% of cisplatin was complexed with NaHA, thus only 24% of cisPt was immediately available. It could be that the high molecular weight of NAHA used hindered cisplatin uptake by cells. Therefore, expanding on the effect of HA molecular weight, the complex was re-formulated using LMW HA and was applied for 4 and 24 hours only at 2 and 10µM cisPt concentrations. The results are reported in Figure 1.

Table 1. *IC_{50} (µM) of CisPt, CisPt/NaHA and CisPt+VA in $BxPC_3$, MiaPaCa-2, A375, A549.*

	IC_{50} (µM)		
Cell lines	**cisPt**	**cisPt/NaHA (HMW)**	**cisPt+VA**
$BxCP_3$	9.63 ± 0.05	10.58 ± 0.08	ND
MiaPaCa-2	5.04 ± 0.04	5.03 ± 0.11	5.11 ± 0.05
A375	2.17 ± 0.01	2.32 ± 0.04	2.39 ± 0.06
A459	4.22 ± 0.10	4.31 ± 0.07	4.28 ± 0.05

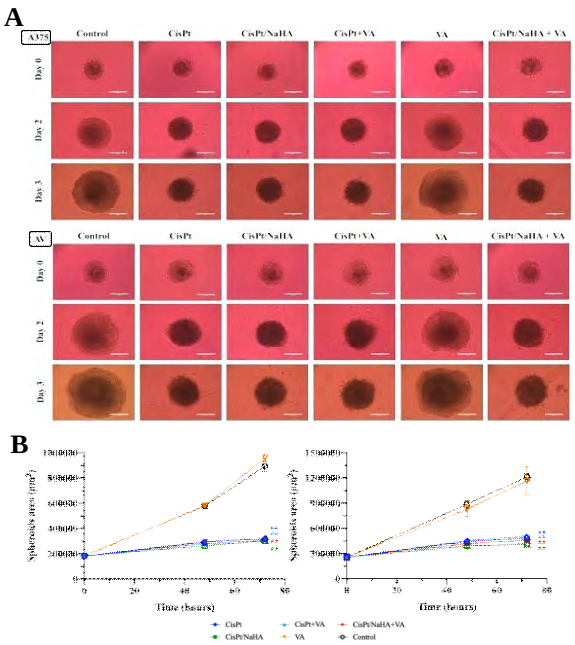
Figure 1. *In vitro cytotoxicity of cisplatin (cisPt), cisplatin/hyaluronan (cisPt/NaHA) complex and cisplatin plus valproic acid (cisPt+VA) in the following cell lines: A375, AV, MiaPaCa-2 and A549.*



It is important to mind that in case of the complex, coordinated CisPt may not be immediately available for cell uptake. In A459 cell line a greater efficacy of the complex was observed, not seen before with HMW NaHA. Similarly, in A375 cell line we observed a significant cytotoxic effect with cisPt/NaHA complex in comparison with cisplatin alone, both after 4 and 24 hours of treatment exposure. Moreover, this cell line was sensitive to the addition of valproic acid. Based on the above, we selected the melanoma cell line (A375 and AV, the resistant one) for further study with 3D multicellular melanoma spheroids. In this we observed

significant reduction in the area of spheroids for the cisPt/NaHA complex and for the cisPt/NaHA complex plus valproic acid groups in comparison with control. Spheroids surface in cisPt/NaHA complex group exhibited rough morphology indicating its cytotoxic effect while control spheroids were continuously growing and exhibited a smooth and uniform surface. This different morphology and significant reduction in area of spheroids associated with the cisPt/NaHA complex and cisPt/NaHA plus valproic acid treatment groups indicate their strong antitumor efficacy as compared to cisplatin alone in vitro (Figure 2).

Figure 2. *(A) Representative images of spheroids treated with control, CisPt, CisPt/NaHA, CisPt+VA, VA and CisPt/NaHA+VA on days 0-3 of treatment in A375 (upper) and in resistant melanoma (bottom). (B) Comparison of the area of spheroids (A375 upper; resistant melanoma bottom) treated with different treatment groups during the 72 hours of treatment. (*p < 0.05, **p < 0.01, ***p<0.001, ****p < 0.0001)*



Conclusions

Overall, this study confirms the efficacy of CisPt/NaHA complex in all the cell lines tested and the possibility to make a combination with valproic acid in order to increase efficacy. These preliminary results open to the next study of CisPt/NaHA complex, delivered in gel form to a murine model of melanoma. The gel form allows to inject the complex, possibly alsowith valproic acid, directly in the mass tumor.

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POSTER N. 11

FORMULATIVE STRATEGIES FOR THE BIOAVAILABILITY IMPROVEMENT OF DRUGS WITH POOR SOLUBILITY/PERMEABILITY

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Drugs belonging to BCS II-IV classes present critical bioavailability related to either solubility limitations or poor permeability through absorption epithelia.
These drugs represent an important challenge in preformulation and formulation studies stimulating the research of different solutions and strategies.
Among these, the research of our group is mainly involved in the following approaches:

1) development/characterization of solid-state modifications such as polymorphism, solvato-morphism and amorphism [1], solid dispersions and drug-cyclodextrin complexes [2]
2) polymeric micelles and nanoemulsions based on hydrophobically modified bioactive polymers
3) development of nanocrystals especially based on amphiphilic bioactive polymers as stabilizers.
The group is also involved in the *in-vitro* biological characterization of the interaction of actives and drug delivery systems (DDS) with cell and tissue models and permeability studies.

Among amphiphilic bioactive polymers, some work has been performed in the last few years about hydrophobic derivatives of hyaluronic acid (HA) for nanosystem development; as an example, HA hexadecylamine, was used for mucosal delivery of the antifungal drug clotrimazole (CLO), whose concentration could be increased about 36-fold compared to the drug inherent aqueous solubility [3]. However, most of the work involved the study of chitosan (CS) derivatives obtained by the ionic interaction of this polysaccharide with fatty acids. In particular, the interaction of CS with oleic acid (OA) results in the polysaccharide chitosan oleate (CS-OA) that presents a peculiar ability to stabilize O/W emulsions and nanoemulsions (NE) having as oil phase either essential oils or organic solvents [4].
The stabilization of ethyl-acetate or $CHCl_3$ NEs with CS-OA results in the one-step preparation of nanoparticles (NPs) based on biodegradable polymers (for example PLGA) coated with a shell of CS-OA in which CS is anchored to the PLGA core thanks to oleic acid moieties, while the hydrophilic chains form the shell towards the aqueous environment.

The resulting strong positive charge of the NPs can be exploited for the ionic adsorption of counterions, that in turn modify the NPs surface. This mechanism was exploited for the association of NPs with a fluorescent cyanine (ICG) for photo dynamic therapy (PDT) application. Although the sensitivity of the ionic interaction to medium dilution suggests limiting the use of the ICG NPs for loco-regional delivery, when ICG was associated with NPs the irradiation always produced a statistically significant reduction in Caco-2 cell viability compared with the irradiated ICG solution. This can be explained by easierinternalization of NPs by endocytosis and higher concentrations of ICG associated with NPs [5].

Both in NE and in NPs, the shell maintains some of the properties that make CS particularly interesting in drug delivery, such as mucoadhesion, positive charge and interaction with cell membranes, absorption promotion, anti-infective and immunomodulatory activity. Someof these effects are supported by the presence of OA, which also participates with anti-infective behavior and absorption promotion properties.

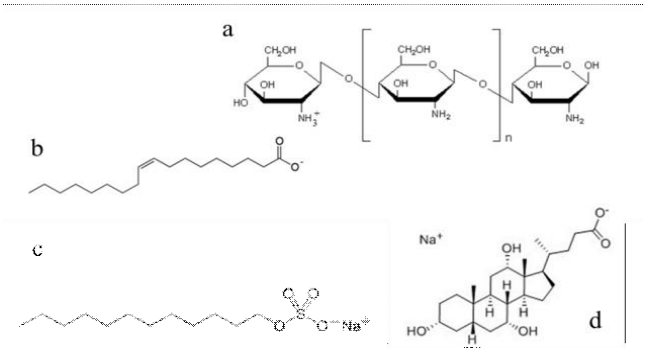

Figure 1. Chitosan (a) and oleic acid (b), Na dodecyl sulphate (c) and Na Cholate (d), studied as counterions for hydrophobic modifications

More recently, hydrophobically-modified CSs have been studied as stabilizers for nanosuspensions, obtained by wet milling, of apremilast, an anti-inflammatory drug PDE4 inhibitor, BCS class IV, authorized for psoriasis treatment. A nanosuspension sample was obtained by using Lutrol F127, well established as a stabilizer in wet milling, for comparison purposes. Quite differently from what observed in the case of NEs, CS-OA resulted not suitable to stabilize the apremilast nanosuspension, while nanocrystals stabilized with chitosan association with Na dodecyl sulphate (CS-SDS) or Na cholate (CS-cholate) were obtained with dimensions slightly higher than 400 nm after only 6 hours milling.

The ratio between CS and the two anionic surfactants has been studied, with the aim of obtaining a positive total charge of the nanoparticles. The prevalence of CS at the NPs surface is in fact relevant for the mucoadhesion behavior that was confirmed *in vitro*, after the addition of a mucin solution, by the decreaseof zeta potential from positive to negative values andby the decrease of non-adsorbed mucin quantified by Schiff reaction.

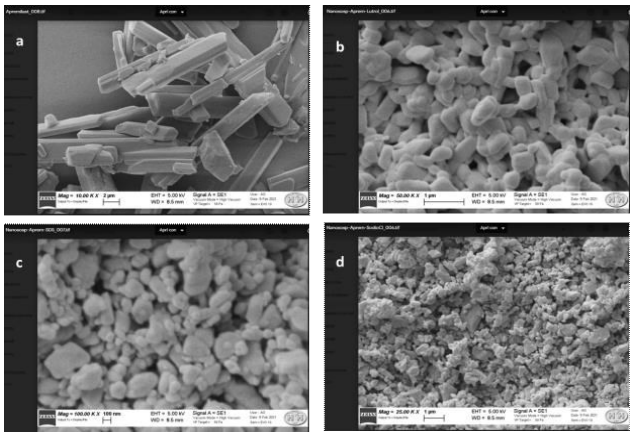

Figure 2. SEM pictures of apremilast (a) and of the samples subject to wet milling with Lutrol F127 (b), CS-SDS (c) and CS-Cholate (d)

After 20 days of conservation at 4°C the sample stabilized with CS-cholate maintained dimensions comparable to the initial ones (from 473±4 nm to 457±70 nm) while for CS-SDS a clear increase of dimensions was observed (from 453±30 nm to 799±11 nm), indicating less efficient stabilization in this case.
The shift of the melting temperature of Apremilast to slightly lower values with respect to that of the pure substance is observed for all the nanosuspensions and can be attributed to the intimate mixing of the two components and to the reduction of dimensions to the nanometers range. The decrease in the specific enthalpy of melting of the drug recorded for these systems could also be attributable to a slight loss of crystallinity during the formation of the nanosystem. FTIR and X-ray analysis supported this possible explanation.
SEM pictures (Figure 2) show the reduction in dimensions that occurred for the two samples and for the drug subject to nanomilling in presence of Lutrol F127, and of the two CS derivatives.
The dissolution profiles of CS-cholate and F127 nanosuspensions showed a clear improvement in the dissolution rate with respect to the active and its physical mixtures with the excipients (over 80% in 15 minutes vs less than 40%).

Cytocompatibility was evaluated on fibroblasts and adenocarcinoma human alveolar basal epithelial cells (A549). For 5 µM concentration for all the samples the viability was over 80% in all cell lines, except for CS-cholate in A549, that anyway resulted in 70 % viability, indicating the possibility of nanosuspension application for topical delivery.

Special thanks from the group are due to Prof C. Caramella and Prof G. Bettinetti for their valuable teachings.

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POSTER N. 12

SURFACE FUNCTIONALISED PARENTERAL NANOEMULSIONS FOR ACTIVE AND HOMOTYPIC TARGETING TO MELANOMA

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Targeted therapies and immunotherapies are currently practiced against melanoma, specifically as adjuvant in high-risk stage IIB/C (primary cutaneous tumour thickness > 2.0 mm) and stage III (with metastasised lymph nodes), and as advanced treatment in stage IV (metastatic). Indeed, since nearly half of the human melanomas possess a V-RAF murine sarcoma viral oncogene homolog B (BRAF) mutation, BRAF and Mitogen-activated protein kinase (MEK) inhibitors are used for targeted therapies, but with the relevant limitation of chemo-resistance. Therefore, advanced melanoma still represents a life threat, driving to the development of tumour targeted nanotechnology approaches, with the aim of increasing therapeutic efficacy and reducing drug doses employed, as well as unwanted side effects [1]. Within this concern, injectable lipid nanoemulsions (IL) can be functionalised with targeting proteins, such asantibodies or other ligands for specific antigens over- expressed on melanoma cells. Indeed, their biocompatibility and favourable technological features including tuneable size and surface charge, make them capable to cross biological barriers, prevent drug chemical and/or biological degradation, and slower drug clearance from the bloodstream [2]. For this purpose, two different strategies can be followed, by exploiting the targeting potential of endogenous proteins. The first one is obtained by chemical grafting onto IL surface of Transferrin (TRF) as the specific protein ligand for active targeting. Indeed, TRF shows a relatively high interspecies equivalence with respect to receptor binding [3] and its receptor is over- expressed in melanoma [4]. IL can be successfully conjugated to TRF by using maleimide-based linkers [5]. In an alternative approach, protein-enriched cell membrane fragments (CMF) obtained from melanoma cells can be used to wrap IL surface, owing to their excellent properties, such as long blood circulation, immune escape, and homotypic targeting ability.

In this experimental work, IL were functionalised with proteins, either by means of chemically grafted TRFfor active targeting, or by CMF wrapping for homotypic targeting. Physico-chemical characterisation of targeted IL was performed, including western blot analysis of CMF proteins used to wrap IL. Surface functionalization was easily achieved thanks to IL physico-chemical stability and sterile filterability, allowing interface reactions, and wrapping by extrusion, without alteration of the mean droplet size. Moreover, the targeting efficiency was preliminarily evaluated using flow cytometry internalisation studies in two-dimensional melanoma cellular models, after labelling of formulations with the fluorescent probe 6-coumarin. Despite both the targeting approaches were suitable to increase internalisation with respect to unfunctionalized IL, stronger evidence came for CMF-wrapped formulations, since TRF targeting efficiency might be limited by competition with the endogenous protein. Moreover, in the case of TRF, a more pronounced internalisation was achieved when a pegylated heterodimer was employed for conjugation ($p < 0.05$).

In perspective, given the promising results obtained, such functionalisation approaches might be translated to IL loaded with drugs or drug combinations aiming at melanoma therapy.

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POSTER N. 13

A new Antiparasitic Cocrystal of Praziquantel and Niclosamide

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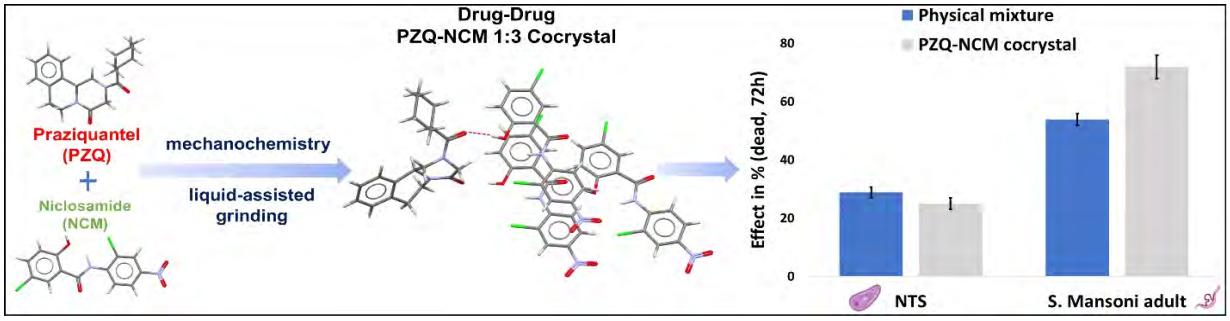
Cocrystallization of two active pharmaceutical ingredients (APIs) (drug-drug cocrystals) has recently emerged as an innovative drug development strategy, representing a new approach for developing multi-drug solid forms [1-2]. It has been already observed that such innovative systems are more advantageous compared to the traditional multimodal therapy, i.e., fixed-dose combinations (FDCs), the latter presenting important stability issues and differences of solubilization kinetics among the combined drugs [3].



Drug-drug cocrystals instead are unique stable crystalline solid phases that can positively affect the pharmacokinetic and pharmacodynamic parameters of the drugs forming the cocrystal [1-2].
Importantly, drug-drug cocrystals may allow coadministration of two APIs thus providing a simplification of the drug dosage and a subsequent improvement of patient compliance and decreasing the risk of side effects or drug resistance phenomena [3].
In this context, we report the successful cocrystallization of Praziquantel (PZQ) and Niclosamide (NCM) to obtain an API-API antiparasitic cocrystal.
Praziquantel (PZQ) is the anthelminthic drug of first choice in the treatment of Schistosomiasis, that is an infection caused by trematodes of the genus Schistosoma [4], while Niclosamide (NCM) is mainly applied to cure parasite infestations, such as those of tapeworms and cestode [5].
Specifically, the novel solid is formed by PZQ and NCM in a 1:3 molar ratio, and it can be obtained in only 120 min through a sustainable one-step mechanochemical process in the presence of micromolar amounts of methanol.
Synchrotron single crystal measurement attested that the new system crystallizes in the monoclinic space group of $P2_1/c$, showing one PZQ and three NCM molecules linked through homo- and heteromolecular hydrogen bonds in the asymmetric unit. This result has been also confirmed by SSNMR and FT-IR data. PZQ-NCM cocrystal exhibits a plate-like habitus, as evident from SEM analysis, and a melting point of 202.89 °C, which is intermediate to those of the parent compounds.
Spectrometric evaluations have also been carried out to understand whether PZQ undergoes chemical degradation when ground in the presence of NCM upon LAG conditions. From literature is well-known the degradation tendency of PZQ in binary ground mixes with the insurgence of peculiar degradation products as a function of the excipient used [6-7-8]. In the presence of NCM, no evidence of PZQ degradation is noticeable and the cocrystal shows a very stable profile under several conditions.
In the context of physical stability in aqueous solution, the new multicomponent system prevents NCM transformation into the well-known insoluble monohydrate [9]. The solid-state features of the cocrystal remain unchanged over a period of 12 months at ambient temperature, with no signs of dissociation in the parent compounds or transition in NCM monohydrate, typically insurging within one month of storage at ambient conditions.
Finally, the new PZQ-NCM cocrystal exhibits higher anthelmintic activity (%-effect of activity reduction) against in vitro S. *mansoni* models compared to the simple physical mixture of the two, thus resulting a very promising system for future in vivo studies.

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POSTER N. 14

COMBINATIONS OF RESINS AND ESSENTIAL OILS FOR SKIN DELIVERY

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This study is a part of the European Project EthnoHERBS, which aims to develop an efficient platform for discovering novel therapeutic agentsagainst skin disorders, relying on the great potential of traditional medicine and the rich biodiversity of the Flora of the Balkan peninsula. In this research, four plant species have been selected because of their great interest in the ethnomedicine related to skin diseases: Origanum *dictamnus* L. (Lamiaceae, Cretan Dittany), *Salvia fruticosa* L. Mill. (Lamiaceae, Greek Sage) [1], *Pistacia lentiscus* L. (Anacardiaceae, Mastic Tree) [2], and *Cistus creticus* L. subsp. *creticus* (Cistaceae, Pink Rockrose/Ladano) [3].

The essential oils (EO) from O. dictamnus and S. fruticosa were isolated by steam distillation, while the resins were collected directly from the plants. The resin of C. creticus was purified using diethyl ether. GC and HPLC analyses were performed to obtain the fingerprints of EOs and resins. The main compounds were found to be carvacrol and eucalyptol. These EOs and resins show antibacterial, antiviral, and antifungal activity, but also antioxidant and anti-inflammatory properties.

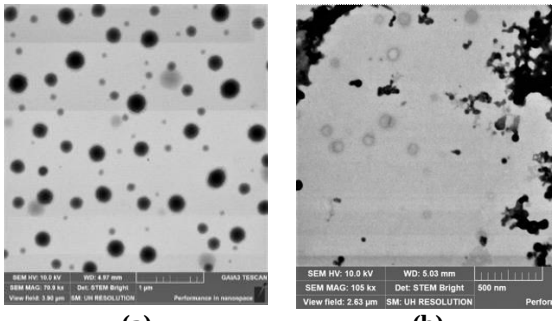
Due to the high chemical instability and volatility of the constituents of both raw materials and the low solubility of the latter, a proper dosage form was necessary to preserve single components and optimize their dermal bioavailability. For this purpose, we developed nanovesicles loading a combination of an EO and a resin to merge the biological activity of the two extracts.

Nanovesicles made of phosphatidylcholine and cholesterol were prepared by the thin layer evaporation method, according to previous studies on essential oils' formulation [4,5], to combine in a unique formulation *O. dictamnus* EO and *C. creticus* resin (Figure 1a). By varying one factor at a time, we selected the excipients' concentrations and ratios. We included 3% v/v Tween 80 in the hydration medium to stabilize the vesicle's bilayer. The obtained nanovesicles had sizes of 101.3±1.035 nm and a polydispersity index (PdI) of 0.2101±0.008457. Preliminary studies on encapsulation efficiency revealed an essential oil entrapment of 96.07±2.477 %, determined as entrapped carvacrol, which was selected as a marker for the quantitative analysis, being the most abundant EO component.

The *S. fruticosa* EO and *P. lentiscus* resin were combined in nanovesicles prepared using only phosphatidylcholine by the thin layer evaporation method, exploring the possibility of using the resin as a vesicle bilayer component, imparting a potential biological activity at the nanocarrier itself (Figure 1b). In the beginning, we tested different gravimetric ratios between phosphatidylcholine and resin: 3:1(2), 4.5:1(2), 6:1(2), and 7.5:1(2). The formulation prepared with the 6:1 was found to be the most promising in terms of sizes (121.7±1.352) and PdI (0.171±0.006009) and was loaded with *S. fruticosa* EO. Tween20 (3% v/v) and Brij20 (1% w/v) were added to the hydration medium as the stabilizers of the vesicle's bilayer to avoid the ultrasonication process to homogenize the nanovesicles' diameter.

All nanovesicles were found to be stable over a month. Release studies and the genotoxicity assay are still ongoing. The latter is being carried out on *Schizosacharomyces pombe* wild type 972h to assess the protective effect of formulated EOs and resins, alone and combined, against genotoxic agents. By contrast, preliminary results evidenced a strong antifungal activity of *C. creticus*.

In conclusion, formulating EOs and resins in stable dosage forms is still mandatory for improving their therapeutic use. Further studies will assess the effectiveness of the formulations for a medical or cosmetic application.



(a) **(b)**

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POSTER N. 15

NATURAL DEEP EUTECTIC SOLVENTS: A PROMISING TOOL FOR IMPROVING THE BIOAVAILABILITY OF A BCS CLASS IV API

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The formulation of BCS class IV drugs represents one of the major challenges for pharmaceutical technologists. The main reasons are related to their poor water solubility and low permeability through gastrointestinal membranes. Benznidazole (BNZ) is the drug of choice for the treatment of the acute phase in Chagas disease; however, high dosages and very long times of treatment are required since it belongs to BCS class IV. For this reason, it is important to increase both thesolubility and permeability of BNZ. In recent years, several technological approaches have been studied for this purpose [1,2] and all of them having pros and cons. Recently, Natural Deep Eutectic Solvents (NaDES) have emerged as potential green approach for solubility enhancement of BCS class II APIs [3]. NaDES are eutectic mixtures of two or more natural components which, when present in a specific ratio, melt at a considerably lower temperature than the melting temperature of the individual substances following the establishment of hydrogen bonds between a donor (HBD) and an acceptor (HBA).

This project aims to evaluate the use of NaDES as an innovative approach for the development of BNZ formulations with improved biopharmaceutical properties. Different NaDES formulations were prepared and the solubility enhancement of BNZ was evaluated. Then, NaDES were formulated as gels and their release profiles and cytotoxicity were assessed.

A screening of several NaDES formulations revealed that the highest solubility values of BNZ were obtained in NaDES composed by choline chloride and organic acids (citric and malic) (**Table 1**), with an increase of about 30 times compared to the solubility value in water (201.95 ± 18,41 ug/mL) after 24 h at room temperature.

NaDES	HBA	HBD	Ratio w/w	Added H_2O (% w/w)
1	Choline chloride	Citric acid	3:1	10
2		Citric acid	2:1	7.5
3		Malic acid	1:1	0

Table 1 Composition of the selected NaDES prepared by heating at 90°C

These eutectic mixtures were used to prepare hydrophilic gels using xanthan gum and a suitable amount of water.

Then, release profiles of the gels were performed by simulating the passage of the drug along the buccal (SSF, pH 6.8) and gastrointestinal tract (SGF, pH 1.2 and SIF, pH 6.8). The results showed a significant increase of the release of BNZ contained in the eutectogel compared to the dissolution profile of the pure drug, reaching a condition of supersaturation already after 5 min. Supersaturation was maintained in the gastric fluid and even (except for gel NaDES 3) in the intestinal one (**Figure 1**).

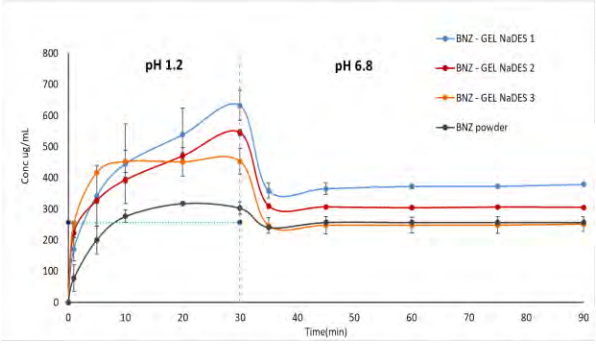

Figure 1 Dissolution profiles of eutectogels compared with BNZ powder in SGF pH 1.2 and SIFpH 6.8. The dotted green line indicates the equilibrium solubility of BNZ (257.26 ± 3.20 µg/mL) in pH=1.2 at 37°C.

Subsequently, to evaluate the biocompatibility of the gel samples, cytotoxicity tests were performed on gingival fibroblasts. Despite the low pH (2-2.5), the data obtained for eutectogels showed overall a good cell viability with respect to the gels prepared without the NaDES formulations.

Conclusion and perspectives

The data obtained show that the selected NaDES were able to improve in vitro the biopharmaceutical properties of BNZ. Pharmacokinetic studies on rats are currently ongoing to prove the efficacy in promoting drug adsorption.

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POSTER N. 16

NASAL ADMINISTRATION OF MICROENCAPSULATED DIMERIC CONJUGATE OF FERULIC ACID: TOWARDS A NEW APPROACH FOR NEURODEGENERATIVE DISEASES

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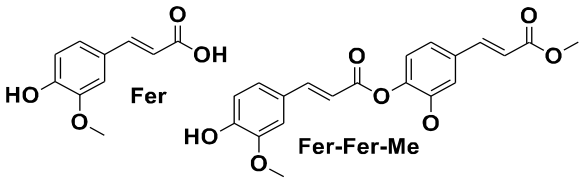
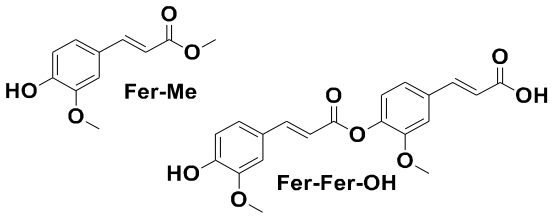
Ferulic acid (Fer) displays antioxidant and anti-inflammatory properties, being a reactive oxygen species (ROS) scavenger and a down regulator of NF-κB signalling, respectively. Due to these properties,Fer induces an inhibition of pro-inflammatory cytokines (*e.g.* TNF-α), and has been proposed for the prevention and therapy of neuroinflammatory disorders, such as neurodegenerative diseases [1]. Despite Fer is rapidly absorbed following oral administration and permeates the central nervoussystem (CNS) from the bloodstream, its permanence in the brain appears low and often inadequate for therapeutic proposals [2]. Aiming to increase the uptake and residence time of Fer in the CNS, a prodrugapproach is proposed. Specifically, the prodrug has been constructed according to a dimer design, in order to increase the Fer loading on solid lipid microparticles (SLMs) designed as nasal formulation. The carboxylic moiety of a molecule of Fer was therefore esterified with the phenolic group of another Fer molecule, obtaining an ester conjugate (Fer-Fer-OH) in the absence of linkers, which avoid the production of unwanted subproducts when hydrolysed in physiological environments. Finally, the dimeric conjugate of Fer was methylated on the carboxylic moiety (Fer-Fer-Me) in order to increase its lipophilicity and potential loading in SLMs.



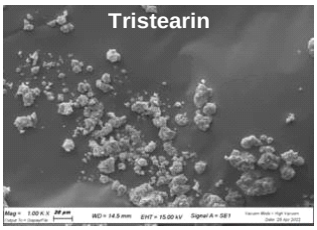
Fer-Fer-Me was characterized by evaluating, via HPLC-UV, its potential prodrug behaviour in physiologic fluids, such as rat whole blood or brain homogenate. Moreover, the potential antioxidant and anti-inflammatory activities of Fer-Fer-Me itself were investigated, by the DPPH assay [3] and inhibition of TNF-α production as inflammatory response by PC12 cells, respectively. SLMs based on tristearin or stearic acid were loaded with Fer-Fer-Me and characterized by scanning electron microscopy (SEM), particle size, powder X-ray diffraction (PXRD), differential scanning calorimetry (DSC), thermogravimetric (TG) and FTIR analysis. The ability of the SLMs to modulate the dissolution or release rate of Fer-Fer-Me in aqueous environments, in comparison to the dissolution rate of Fer, was investigated. The results of these studies allowed to select stearic acid-based SLMs for nasal administration of Fer-Fer-Me to induce the brain targeting of the prodrug.

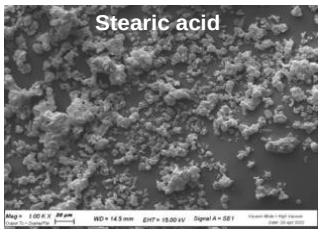
Fer-Fer-Me exhibited an antioxidant power about the 70% compared to that of Fer; moreover, it appeared able to induce anti-inflammatory effects on PC12 cells, appearing itself as a promising compound for the treatment of neurodegenerative disorders. Fer-Fer-Me was degraded in the whole blood or brain homogenates of rats, allowing the concomitant appearance of the products derived by the hydrolysis of its methyl-ester group (Fer-Fer-OH) or its ester-conjugation (Fer-Me and Fer).



Fer-Me is known to induce antioxidant and anti-inflammatory properties similar to that of Fer [4].

The hot emulsion technique allowed to obtain tristearin or stearic acid SLMs loaded with Fer-Fer-Me, as can be seen from the SEM images below.





The particles evidenced the aptitude to form aggregates with sizes in the 100 μM range. PXRD revealed the poorly crystalline nature of the samples and a partial interaction between Fer-Fer-Me and lipids in loaded SLMs. Thermal analysis (DSC and TG) revealed that the Fer-Fer-Me was well dispersed in the lipid matrix. FTIR spectra suggested an interaction between the Fer- Fer-Me and the lipid phase supporting the thermal data.The amounts of Fer-Fer-Me encapsulated in tristearin or stearic acid based microparticles were 0.488 ±

0.005% and 1.87 ± 0.05%, respectively, which corresponded to encapsulation efficiencies of 18.75 ± 0.19% and 72.14 ± 2.10%, respectively. These values appear significantly higher in comparison to those of Fer loading in the same types of SLMs [4].

The dissolution studies of Fer and Fer-Fer-Me and the release studies of Fer-Fer-Me from the SLMs were performed at 37°C in a mixture of water and methanol (70:30 v/v). Fer-Fer-Me was characterized by a very poor dissolution rate, being solubilized about the 8% of the total raw powder amount after six hours of incubation. The tristearin based SLMs showed a release pattern characterized by a burst effect of about 10% of the incorporated Fer-Fer-Me, followed by a relatively slow release (about 30% of encapsulated prodrug released within 6 h). The stearic acid based SLMs, despite the relatively high encapsulation efficiency, showed a Fer-Fer-Me release pattern characterized by a burst effect of about 50%, followed by a relatively fast release allowing to obtain the dissolution of more than 80% of the loaded prodrug within 6 hours of incubation.

The SLMs based on stearic acid were therefore characterized not only by a satisfactory encapsulation efficiency but also by their ability to induce a fast dissolution of Fer-Fer-Me in comparison to the raw powder. Thus, these microparticles were selected for nasal administration of the prodrug, in order to verify its potential uptake in the CNS. Fer and Fer-Fer-Me were firstly administered by intravenous route (1 mg/kg) in order to evaluate and compare their pharmacokinetic profile in the bloodstream and cerebrospinal fluid (CSF) of rats; then Fer-Fer-Me loaded in SLMs was nasally administered (1 mg/kg) and the pharmacokinetic profiles obtained in the bloodstream and CSF of rats were compared with those obtained by the nasal administration of Fer (1 mg/kg).

The bloodstream half-life of intravenously administered Fer was 20.3 ± 1.3 minutes, with an area under concentration/time curve (AUC) of 244 ± 13 $\mu g \cdot mL^{-1} \cdot min$. The CSF AUC value of the Fer profile (which decreased to zero within 120 min) was 3.3 ± 0.3 $\mu g \cdot mL^{-1} \cdot min$. The half-life in the bloodstream of intravenously administered Fer-Fer-Me was 18.0 ± 1.9 minutes; moreover, relevant amounts of Fer-Me and Fer derived by the hydrolysis of the prodrug were evidenced. The AUC value of the Fer-Fer-Me profile in CSF of rats was 9.8 ± 0.5 $\mu g \cdot mL^{-1} \cdot min$. These results indicate that both Fer and its prodrug Fer-Fer-Me evidence the aptitude to permeate in the CNS of rats from the bloodstream.

Following its nasal administration, Fer was detected during time both in the bloodstream and CSF of rats. The AUC value in the bloodstream was 99.1 ± 4.8 $\mu g \cdot mL^{-1} \cdot min$, allowing to calculate a nasal bioavailability surprisingly high, being its value 40.5 ± 2.8 %. This result seems to confirm the marked ability of Fer to permeate across the physiologic barriers, as evidenced by its aptitude to permeate in the CNS from the bloodstream. The AUC value of the Fer profile in CSF of rats was 5.16 ± 0.2 $\mu g \cdot mL^{-1} \cdot min$, slightly higher than that obtained by intravenous administration of Fer but lower than that of the prodrug Fer-Fer-Me intravenously administered at the same dose.

The nasal administration of Fer-Fer-Me (1 mg/kg) as water suspension did not allow to obtain its detection neither in the bloodstream, nor in the CSF of rats, similarly as its hydrolysis products. This behavior can be attribute to the very low dissolution rate of Fer-Fer-Me in aqueous environments. On the other hand, the nasal administration of a same dose of this prodrug encapsulated in stearic acid based SLMs allowed its detection in both bloodstream and CSF of rats. The profiles of Fer-Fer-Me and its hydrolysis products in the bloodstream suggest that the nasal bioavailability ofthe prodrug is relatively high. Indeed, the sum of the AUC values corresponding to the profiles of all compounds detected in the bloodstream, following intravenous and nasal administration were 560 ± 20 $\mu g \cdot mL^{-1} \cdot min$ and 348 ± 16 $\mu g \cdot mL^{-1} \cdot min$, respectively. Concerning the CSF, the AUC value of the Fer-Fer-Me profile was 108.5 ± 3.9 $\mu g \cdot mL^{-1} \cdot min$, *i.e.* about 20 times higher than that obtained by nasal administration of a same dose of Fer, or about 10 time higher than that obtained by the intravenous administration of the prodrug itself; moreover, at 120 minutes Fer-Fer-Me was still quantifiable.

Overall, the results indicate that the nasal administration of the stearic acid SLMs loaded with Fer-Fer-Me is sensibly efficacious in the prodrug brain targeting, by enhancing both amounts and permanence in the rat CSF, in comparison to Fer. This formulation appears therefore promising for the treatment of neurodegenerative disorders, taking into account the antioxidant and anti-inflammatory activities of the prodrug itself and its ability to be hydrolyzed in central environments to Fer and Fer-Me, which are both known to induce neuroprotective effects [4].

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POSTER N. 17

SILK FIBROIN BIOINK IN TENDON REGENERATION: 3D PRINTED MATRIX FOR CONTROLLED RELEASE OF MSC EXTRACELLULAR VESICLES

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Sodium alginate (SA)-based hydrogels are often employed as bioink for three-dimensional (3D) scaffold bioprinting [1]. They offer a suitable environment for cell proliferation and differentiation during tissue regeneration and control the release of growth factors and mesenchymal stem cell secretome [2], which is useful for scaffold biointegration [3]. However, such hydrogels show poor mechanical properties, fast-release kinetics, and low biological performance, hampering their successful clinical application. In this work, silk fibroin (SF), a protein extracted from *Bombyx mori* cocoons, with excellent biomechanical properties and frequently used for controlled drug release, was blended with SA to obtain improved bioink and scaffold properties.

Regarding its ideal characteristics, the SA-SF bioink has to be easily printable with good mechanical properties. Therefore, considering that SF has different conformations, ideally, SF must: (A) remain in Silk I conformation (a mix of a random coil, α-helix domains, and β-turn structures), i.e., in solution, duringprinting to prevent the nozzle from clogging; (B) after being printed, the conformational change from Silk I toSilk II (abundant β-sheet structures) must be induced toobtain a material with higher tensile strength and modulus.

Point A was resolved by changing the standard degumming process (i.e., the extraction of SF) and, specifically, by increasing the boiling time of cocoons in 0.02 M Na_2CO_3 from 30 min to 1, 2, and 4 h. Following dissolution in 9.3 M LiBr, SF degummed for1, 2, and 4 h was printable without any clotting of the needle; this was due to the decreased molecular weight (MW) of SF, as revealed by SEC-UV analyses. Concerning point B, even SF degummed for 1, 2, and 4h was able to change the conformation from Silk I to Silk when treated with 20% w/v KCl (according to what happens in the silkworm gland), as revealed by physical-chemical characterization.

Then, the SA-SF blends' printability and shape fidelity were demonstrated. The printability of SF degummed 1 h was maintained for up to 7 days, while for SF degummed 2 or 4 h, it was extended for up to 14 days, even if a reduction in printing performance was observed. A good shape fidelity in the pores was observed, while the material's viscous and potentially collapsible nature was highlighted.

The mechanical characterization of the printed hydrogels was also performed. The degumming process did not affect the compression response for the SA-SF formulations and their compressive moduli were three times higher than that of the hydrogel formed with only SA. Conversely, no influence on tensile response was detected.

Finally, the release profile of lyosecretome – a freeze-dried formulation of MSC-secretome containing extracellular vesicles (EVs) – from SA-SF bioinks was determined. The addition of SF significantly modified the release of EVs with respect to the baseline of SA-only hydrogel, which was considered a control, depending on the degumming time. Specifically, the release of EVs was faster than the control when 1 h degummed SF was used, while the use of SF degummed for 2 or 4 h significantly slowed the release.In detail, after 2 h, SA-SF-1h had a burst release of almost 50% vs. 30% for the control, and 16% and 11% for SA-SF-2h and SA-SF-4h, respectively.

SF also modified the kinetics and mechanism release: SA-SF hydrogels can control the release of EVs by a combination of diffusion and erosion, and the diffusion speed decreases as the degumming time of SF increases. This is likely due to the increased compactness of the polymeric network: the low MW ofSF degummed 2 or 4 h does not affect the interactions among polymer chains.

These results lay the foundation for developing SA-SF bioinks with modulable mechanical and EV-release properties and their application in 3D scaffold printing.

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POSTER N. 18

SILK FIBROIN NANOPARTICLES FUNCTIONALIZED WITH cRGD FOR ACTIVE DRUG TARGETING IN CANCER THERAPY

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Developing drug delivery systems to target cytotoxic drugs directly into tumor cells is still a compelling need with regard to reducing side effects and improving the efficacy of cancer chemotherapy. In this work, silk fibroin nanoparticles (SFNs) have been designed to encapsulate and deliver NDI-1, a highly cytotoxic tetrasubstituted naphthalene diimidederivative that disrupts the cell cycle by specifically interacting with non-canonical secondary structures of DNA [1]. To this end, a fibroin and NDI-1 solution was desolvated in acetone. SFNs were then functionalized on their surface with cyclic pentapeptides incorporating the Arg-Gly-Asp sequence(cRGDs) to provide active targeting toward glioma celllines that abundantly express 3 and 5 integrin receptors [2]. The functionalization of SFNs with cRGD wasperformed by exploiting an azide-alkyne "click reaction" between the azide group of the RGD-based cyclic pentapeptide and triple bond-functionalizedSFNs carrying an alkyne group on their surface (obtained through forming an amide bond between Lysine residues on SFNs surface and N-hydroxysuccinimide linker endowed with a terminal alkyne moiety) [3].

SFNs and cRGD-SFNs were obtained with a process yield above 75% and were nanometric in size, with a mean diameter around or below 100 nm, and the d_{90} always lower than 150 nm. The fibroin matrix formed during the desolvation process could entrap NDI-1, achieving a loading of 1.2±0.4% w/w, with an encapsulation efficiency above 95%. The functionalization with cRGDs did not change the main characteristics of SFNs: the loading of NDI-1 was preserved (1.2±0.3% w/w), as well as the nanometric size. Conversely, the functionalization of SFNs with cRGDs confers a markedly negative surface charge to the nanoparticles. All samples observed by SEM were highly homogeneous in size and appearance, displaying a round shape and a smooth surface, as also confirmed by TEM. No appreciable differences were observed by cryo-EM on the surface of the nanoparticles following functionalization.

Cytotoxicity and selective targeting were assessed by in vitro tests on human glioma cell lines U373 (highly-expressing integrin subunits) and D384 cell lines (low-expressing integrin subunits in comparison to U373). After 72 h of treatment, unloaded SFNs, either functionalized with cRGD or not, showed cytocompatibility, with a cell metabolic activity close to 100% for both cell lines. Conversely, NDI-1-loaded SFNs (not functionalized with cRGD) were highly cytotoxic on both D384 and U373 cells, reducing the metabolic activity below 30% and 20%, respectively. The toxic effect of SFNs-NDI-1 showed to be slightly lower with respect to the free active, and it was not selective for a specific cell line. This suggests that both cell lines can internalize SFNs-NDI-1 by non-specific mechanisms; then, NDI-1 exerts its cytotoxic effectafter the internalization and degradation of SFNs. Functionalization with cRGD provided selectivity incell uptake, coupling non-specific cell membrane protein–particle interactions to specific and target- mediated uptake. As a result, cRGD-SFNs-NDI-1 was highly cytotoxic on U373 cells, while a significantly weaker effect was observed on D384 cells (a metabolic activity of 22% vs. 58%).

This work, therefore, provides an in vitro proof-of-concept of cRGD-silk fibroin nanoparticles' active site-specific targeting of tumors, paving the way for further in vivo efficacy tests.

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POSTER N. 19

SILK FIBROIN NANOPARTICLES LOADED WITH A RE-CALL ANTIGEN ACT AS A TROJAN HORSE FOR RE-DIRECTING IMMUNITY AGAINST CANCER

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The immune system modulates tumor growth by recognizing tumor-associated antigens (TAAs) expressed by cancer cells [1]. The tumor/immune system relationship may lead to (A) tumor elimination, (B) balance between healthy tissues and tumor, and (C) immune invisibility of the tumor. Immunotherapy aims to shift the tumor/immune system balance to the latter's benefit to prevent the above scenario (C) from occurring. This work exploits silk-fibroin nanoparticles (SFNs) to induce re-directed immunity against tumors by delivering a non-specific tumor antigen in tumor cells (Trojan horse approach), together with an adjuvant suitable for generating a robust immune response: the final aim is to induce an antitumor immune response in cancer patients who already possess immunity for said non-specific tumor antigen (e.g., from a childhood vaccination).

To this end, the non-specific tumor antigen (ovalbumin, OVA) or the adjuvant (the oligonucleotide CpG) were solubilized in fibroin and then desolvated in acetone, obtaining SFNs-OVA and SFNs-CpG, respectively. All SFNs appear round, without apparent aggregates, and with smooth surfaces. The yield percentage was consistently above 70%. For SFNs- OVA, the OVA loading % was 32.7%; for SFNs-CpG, the CpG loading % was 7.36%; the encapsulation efficiency was above 97%. All nanoparticles were less than 150 nm in diameter.

Analysis of cell uptake of SFNs showed that all tested tumor cell lines and dendritic cells efficiently endocytosed the nanoparticles.

In vivo experiments aimed to assess 1) whether SNFs-OVA were able to re-direct toward tumor cells a pre-existing immune response against OVA (a non-tumor antigen); 2) whether SNFs-CpG were able to induce within the tumor microenvironment a robust innate immune response. Concerning point 1), the treatment with SFNs-OVA significantly reduced MB49 bladder cancer growth with respect to CTR (no treatment) and SFNs. Similarly, in the case of the B16 melanoma tumor, the treatment with SFNs-OVA significantly slowed down tumor growth. This effect of SFNs-OVA was associated with tumor infiltration by OVA-specific T lymphocytes: the intratumor SFNs-OVA administration effectively re-calls at the tumor site OVA-specific T cells able to kill tumor cells that have endocytosed OVA-loaded SFNs. Concerning point 2), SFNs-CpG effectively reduced tumor growth in the case of the MB49 cell challenge, and 4 out of 5 treated mice were still alive at the end of the experiment for upto 610 days. Similarly, in the case of the B16 cellchallenge, SFNs-CpG administration significantly reduced tumor growth. These findings suggest that SFNs-CpG induced a robust innate immunity activation within the tumor microenvironment. Indeed, proteomics and systems biology showed that peritumoral treatment with either SFNs-OVA or SFNs- CpG dramatically modified tumor microenvironment with respect to the CTR, mainly involving functional modules and hubs related to angiogenesis, inflammatory mediators, immune function, T complex and serpins expression, redox homeostasis, and energetic metabolism.

Finally, a combination of SNFs-OVA and SFNs-CpG (1:1 physical mixture) was administered to B16 melanoma-challenged mice. As a result, tumor growth was significantly reduced with respect to control mice and mice treated with either SFNs-OVA alone or SFNs-CpG alone. This likely indicates that the associated administration of SNFs-OVA and SNFs- CpG allows combining their mechanisms of action, leading to effective activation of both innate and adaptive immune responses and an additive therapeutic effect.

The Trojan horse approach, highly effective in an aggressive experimental tumor, may become a new paradigm for anticancer immunotherapy since easy, cheap, and universally applicable (any type of cancer and any patient) if common vaccinal antigens are used as an immunogen.

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POSTER N. 20

3D-PRINTED OLIVE LEAF EXTRACT (OLE) BASED FILMS AS WOUND DRESSING: SYNTHESIS AND CHARACTERIZATION

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A wound is defined as a disruption of the skin layer resulting from physical or thermal damage. The physiological response of the organism to an injury is characterized by a complex mechanism of articulated events, regardless of the type of wound, be it acute or chronic, and the extent of the status of the patient. The successful treatment of a wound should ensure that the formation of scar tissue is minimal, the amount of necrotic tissue produced is reduced and microbial invasion is prevented. In recent years many types of medication have been developed with different physical forms, starting materials, and clinicalperformances [1].
Hydrogels are three-dimensional, insoluble, crosslinked polymer networks that can hold large amounts of water and biological fluids in their swollen state and represent an excellent dressing for skin repair. They are highly biocompatible and provide a moist environment for wound healing [2]. Moreover, polymeric hydrogels are one of the most feasible classes of ink materials for creating 3D porousscaffolds. Today 3D printing technology represent the most interesting manufacturing technology. It allows the development of dressings with customized parameters (size, dose, swelling properties, drug release profile) able to improve the patient's life quality and, at the same time, to increase the effectiveness of the therapy. The use of this technique in wound healing provides many advantages and makes possible to avoid several drawbacks of traditional dressings, such as skin irritation, high cost of production, discomfort, or difficulty in the application [3].
The incorporation of biomolecules with antioxidant and antimicrobial properties in different types of wound dressings can prevent skin infections and promote the healing process. Among these, the positive effect of polyphenols in olive leaf extract (OLE) on tissue regeneration is well documented [4].
Here, we reported the development of a 3D-printed hydrogels based on sodium alginate and pectin. Alginate is well known for its high biocompatibility and gelling capacity, making it an essential component for the production of hydrogels. Pectin is a non-toxic heterosaccharide suitable for skin tissue applications because of its amorphous nature [5]. Glycerol was used as plasticizer and an OLE solution was added during the ink's preparation. Semi Solid Extrusion was used as 3D printing technology and the physical andmechanical properties of the printed materials have been tested. The antioxidant and scavenging activities of the obtained patches have been evaluated,confirming the properties of the loaded extract. *In vitro* polyphenols release studies have been carried out to assess their safety, efficacy, and quality. A sustained release of polyphenols was observed, suggesting that hydrogels could be effective, if applied on wounds, in controlling bioactive release.

Based on their physical properties, the developed materials represent a suitable system for the controlled release of OLEs polyphenols and the *in vitro* scratch test and biocompatibility studies showed evidence of the positive effects of 3D-printed dressings on wound healing. The results of this research highlight the possibility of using natural bioink formulations in 3D-printing technology and the positive effect of OLE in accelerating skin wound healing.

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POSTER N. 21

SILK FIBROIN/SPIO NANOPARTICLES FOR LOCOREGIONAL CANCER THERAPY: PRELIMINARY BIODISTRIBUTION IN A MURINE MODEL

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Silk fibroin (SF) nanoparticles (SFNs) are a popular choice for drug delivery, as they are biocompatible and biodegradable, they offer optimal entrapment and remarkable cell uptake and have functional groups that lend themselves to functionalization for active targeting [1, 2]. However, SFNs are not suitable, per sé, for theranostic applications. This point is also relevant regarding the biodistribution of SFNs, which is mandatory for regulatory approval but challenging without proper functionalization with imaging probes. In this work, we equipped SFNs with simultaneous diagnostic and therapeutic functions by incorporating iron oxide (Fe_2O_3), which provides potential applications in magnetic resonance imaging (MRI) and loco-magnetic hyperthermia.

Five formulations of SFNs were prepared by increasing amounts of Fe_2O_3 (SFNs-Fe) into the SF solution and desolvating the final mixture in acetone (containing curcumin, used as a model of a lipophilic drug). SEM and TEM morphological investigations revealed the homogeneous distribution of the combined materials in the final SFNs-Fe formulations. Furthermore, themicro-analytical composition of samples showed consistency between the theoretical and actual loading of Fe, and accordingly, the IR characterization showed more resolved characteristic bands of Fe_2O_3 in SFNs- Fe prepared with higher amounts of Fe.

Interestingly, some physical-chemical properties of the obtained SFNs-Fe changed due to the amount of Fe_2O_3 incorporated. Specifically, the mean diameter and the d_{50} value increased when the amount of Fe_2O_3 incorporated was lowered, except for the formulation with the lowest amount of Fe_2O_3, for which the size decreased. This trend was also revealed regarding curcumin release: lowering the amount of Fe_2O_3 incorporated leads to increased curcumin release,except for the formulation with the lowest amount of Fe, for which a decrease was observed. This suggests that SF precipitation into SFNs following desolvation happens with different behaviors depending on the amount of Fe_2O_3 incorporated in the mixture. To give a more precise explanation, the effect of Fe on the release mechanism of curcumin was further investigated by elaborating the release data with the commonly employed kinetic models (Peppas-Sahlin, Ritger-Peppas, Zero-order, Korsmeyer-Peppas). The addition of Fe_2O_3, and, especially, the amount added, affects the balance of diffusion through the material's porosity or case-II relaxation. In detail, adding Fe_2O_3 increases the curcumin released through diffusion, and it is likely that depending on the different amounts of Fe_2O_3, SF desolvation into nanoparticles happens with different behaviors, leading to an increased (or decreased) compactness of the polymer matrix. It can be speculated that a low (but not too low) amount of Fe_2O_3 induces more compactness of the fibroin matrix (thus hampering the diffusion of curcumin, which is indeed lowered), while a higher amount of Fe_2O_3 allows more diffusion due to reduced compactness of the fibroin matrix.

All the formulations showed to be cytocompatible (MTT test): the encapsulation of curcumin into SFNs reduces its cytotoxic effect, and the incorporation of iron oxide did not affect the cell metabolic activity. Conversely, the amount of iron oxide incorporated in SFNs modified the in vitro uptake by cells: the cellular uptake increased when the amount of Fe_2O_3 incorporated was lowered, except then decrease again for the formulation with the lowest amount of Fe_2O_3. This effect can be the consequence of the different size and porosity of nanoparticles that directly affect the mechanism of uptake. Notably, the uptake of the nanoparticles is almost immediate (2 h), and in that time, only a small amount of the drug (curcumin) is released.

Finally, using MRI, it was possible to detect SFNs-Fe in vivo. The SFNs-Fe formulation with the higher amount of Fe_2O_3 was subcutaneously or intramuscularly injected in the flank of C57bl/6 mice. SFNs-Fe showed a hypointense pattern on the T2*WI sequence, localized to the injected anatomical locations until 24 h post-injection.

Overall, incorporating Fe into SFNs allowed for assessing their biodistribution following locoregional administration, which is also relevant in their clinical translation.

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POSTER N. 22

DEVELOPMENT OF MUCOADHESIVE EMULGEL TO BLOCK VIRAL BINDING AND ENTRY INTO HOST CELLS

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Angiotensin-converting enzyme-2 (ACE2), that is highly expressed on human nasal mucosa, has been evaluated by researchers as the most important target to prevent the entrance of the SARS-CoV-2 virus into the human cell [1]. Nasal sprays based on polymers with gelling and mucoadhesive properties, such as HPMC and Carbopol, have been explored as anactive topical alternative to block viral infection [2]. Lately, oils emulsified in aqueous gels have been proposed as dual controlled nasal drug delivery systems for hydrophobic active compounds that can'tbe solubilized in hydrophilic mucoadhesive networks, useful to prolong residence time on nasal epithelium [3]. To meet these requirements, a barrier nasal spray emulgel loaded with a new antiviral compound (RI26) was developed. Preliminary studies regarded the anti- Covid activity assay of RI26 demonstrated its activity against SARS-CoV-2, acting on the entry mechanism. Solubility studies of RI26 in physiological media and partition coefficient evaluation showed that the compound is practically insoluble in all the aqueous systems, but very soluble in octanol. Three different formulations, loaded with 100 µg/mL of drug and a ratio gel- Macadamia oil of 8-2 were tested. The same amount (0.2 % w/v) of Carbopol® 974P NF but variouspercentages (1, 1.2 and 1.5 % w/v) of Methocel™ K4M (HPMC) were employed. Physical appearance, drug content (%DC, HPLC-DAD and UV analysis), pH(pH meter), viscosity (viscosimeter, spindle n3 and n4, at 25 °C during 2 min at 100 RPM), spreadability (increase of diameter under the stress of a weight of 500 g [4]), mucoadhesive strength (detachment forces [5]), and *in vitro* permeation studies (modified Franz diffusion system [6]) were assessed. All these tests were repeated in triplicate, both for blank and loaded emulgels, during the same day for statistics, but for 28 days for stability evaluations (sample stored at 25°C in amber glass vials). As expected, the differences in percentage of HPMC didn't influence the drug content (%DC > 95%, p> 0.05), the pH (between 3.8 and 3.9, p> 0.05), but affected viscosity (lowest value was obtained for 1% of HPMC, while the needed "nasal" value was achieved with 1.2% of HPMC), spreadability(lowest value obtained for 1.5% of HPMC), and mucoadhesive strength (lowest value obtained for 1% of HPMC). Physical appearance showed a reversible phase separation, that quickly disappeared by manual agitation, for both the blanks and the loaded emulgels, after 1 day and 1 week, respectively. This different behavior should be due to the presence of the active compound that interacts with the two phases, leading to a more stable system, as previously reported for oil-in-water delivery systems [7]. This stability should influence the expected barrier function of the loaded emulgel. The *in vitro* permeation study demonstrated the same ability for the three loaded emulgels to avoid the release of the RI26 during the tested 360 min, confirming that the active barrier holds back the compound on the nasal surface, exerting the antiviral activity. Blank and loaded emulgels containing 1.2 % of HPMC were analyzed by Leica DM6 polarized light microscopy (stack 10x) for clearing up the morphology of the emulgels. No differences were observed since the oil phase resulted well and homogenously distributed in the two samples. Finally, a second anti- Covid activity assay was carried out to compare the activity of the no-formulated drug, with the loaded nasal spray with 1.2% of HPMC, and the blank formulation.

Preliminary data (work in progress) showed that blank emulgel has barrier effect against SARS-CoV-2 and loaded emulgel improves the efficacy of RI26 on entry mechanism of the virus.

Based on these interesting results the developed nasal spray could help to block SARS-CoV-2 virus infection at first stages, and it could be applied as possible preventive treatment for many other viruses preventing the entry mechanism.

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POSTER N. 23

THIOL REACTIVE PEG LINKERS FOR HIGH LOADING ADCs

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A high drug to antibody ratio (DAR) represents a highly demanded attribute for antibody-drug conjugates (ADCs), because it increases the *in vitro* potency of the entire platform. However, most anticancer drugs used in clinics (e.g. maytansinoids, auristatins, etc.) are unsuitable for high DAR ADCs owing to their high hydrophobicity. In fact, such issue might affect the stability of the ADC, inducingaggregation phenomena, and impact the pharmacokinetic profile, resulting in a short clearance and a lower *in vivo* activity of the ADC. To overcome this issue, it has been found that the detrimental effects of payload hydrophobicity can be modulated through linker design [1]. In this work we exploited the hydrophilicity of polyethilene-glycol (PEG) basedlinkers to offset the hydrophobicity of eight molecules of auristatin E (MMAE) linked to the eight interchain native cysteines of trastuzumab, an anti-HER2 antibody. The linkers were based on two discrete PEG chains, of different length, branching out from a single point in the structure containing also a ValCit dipeptidefor enzymatic-controlled drug release. In fact, we have previously observed that this *pendant* PEG conformation is able to shield high drug loads in lysine-linked ADCs [2]. Here, three linkers bearing twoPEG chains each with 4, 8 or 12 ethylene oxide units (hereafter referred to as PEG4, PEG8, PEG12, respectively) were compared to each other in terms of stability under stress conditions, *in vitro* cytotoxicity, *in vivo* pharmacokinetic and antitumor activity of the whole ADCs. The antibody-drug conjugates were obtained through a two-steps synthesis (full reduction of the trastuzumab's disulphide bonds followed by complete conjugation of freed thiols with the linker-drug moiety) with high yields in terms of antibody recovery. When characterized by native hydrophobic interaction chromatography (HIC) analysis, the prepared ADCs appeared highly homogeneous, eluting as a major peak corresponding to the fully conjugated species (DAR8). The same analysis well confirmed that increasing the PEG length (from PEG4 to PEG12) promotes a reduced total apparent hydrophobicity of the entire conjugate. Remarkably, when thermally stressed at 40°C and 60%rH over 4 weeks, the aggregates content decreases as the PEG length increases. This in solution stability study highlighted that all PEG linkers allowed to double the amount of drug loaded while maintaining the same level of aggregation of a non-PEGylated low DAR reference ADC, based on ValCitMMAE moiety, suggesting that incorporation of two PEG chains in a *pendant* conformation could be a helpful tool in order to increase the stability of high drug loaded ADCs. Whentested for the *in vitro* cytotoxicity, all ADCs exhibiteda IC_{50} in the nanomolar range against HER2+ cancer cell lines (SK-OV-3 and SK-BR-3), which proves that PEG length does not influence the ADCs' potency, not hindering both the binding with HER2 and the Val-Cit peptide cleavage by lysosomal enzymes at the sizes tested. Pharmacokinetic studies of the ADCs were performed in BALB/c mice, and the total antibody concentration was monitored over 28 days. These data suggested a clear relationship between PEG length and clearance *in vivo*. In fact, the *in vivo* clearance resulted reduced for the ADCs bearing long PEG chains (PEG8 and PEG12) which can shield the burdensome hydrophobicity of eight MMAE molecules. Remarkably, their *in vivo* clearance is even lower than that of a low DAR ADC prepared with a linker missingany PEG chain. On the contrary, PEG4 seems to be notuseful to mask the hydrophobicity of eight moleculesof auristatin E. *In vivo* antitumor activity was evaluated on a SK-OV-3 xenograft model in SCID mice (*n=3*) treated with DAR8 PEGylated ADCs and the control low DAR ADC no PEG at 2.5 mg/kg injected twice (day 0 and day 15), monitoring the tumor volume over 30 days. The obtained results correlated with the *invivo* clearance of the ADCs. In fact, the highest antitumor activity was observed for the ADCs bearing PEG sufficient long to maximize the *in vivo* exposure. On the contrary, DAR8 ADC based on PEG4, which previously shown an accelerated plasma clearance, shown the worst antitumor effect, demonstrating an inverse correlation between apparent hydrophobicity of the entire conjugates and their *in vivo* efficacy.

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POSTER N. 24

PLGA COATED HYDROXYAPATITE/CALCIUM CARBONATE SCAFFOLDS WITH ANTIMICROBIAL ACTIVITY

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Trauma and disease of bone tissues, resulting in severe pain and disability for a lot of people worldwide and whose treatments have a high economic impact, represent major clinical challenges. Orthopedicimplants are medical devices designed to support a damaged bone, to replace a missing joint or bone and finally to augment bone tissue and, traditionally, autografts, allografts, xenografts and metal implants have been used to repair fractures and other bone defects. However, these substitutes are less than idealas each has its own specific problems and limitations [1]. Another important challenge related to boneimplant is the risk of infection. The implant-relatedinfections remain among the main reasons for failure ofbone implantation [2]. Hence the need to dispose biomaterials as new synthetic bone substitutes with structure, properties and functions similar to physiological bone and able to prevent microbial infections.

Thus, the aim of the present study is to obtain three-dimensional porous scaffolds with antimicrobial activity for supporting bone regeneration and growth in cases of major trauma.

These scaffolds are based on calcium carbonate functionalized with hydroxyapatite [HA]. The attention was given on this material because the hydroxyapatite has been considered for decades an ideal biomaterial for bone repair due to its compositional and crystallographic similarity to bioapatites, but unfortunately, due to its very poor biodegradation capacity, it hinders the growth of new bone tissue. So, to overcome this problem, hydrids of hydroxyapatite and calcium carbonate, which is more easily degraded, have been proposed. The presence of calcium carbonate improves hydroxyapatite biodegradability and facilitates the precipitation of calcium and phosphorus ions [3].

Scaffolds based on two kinds of functionalized calcium carbonates (successively indicated OMP and OMD) with different weight ratios between hydroxyapatite and calcium carbonate (OMP: HA 47%; $CaCO_3$ 53%, OMD: HA 85%; $CaCO_3$ 15%) were prepared with the foam replica method by using polyurethane sponge as template. First an OMP(OMD)/blinder slurry was prepared adding a 2% wt polyvinyl alcohol aqueous solution to the inorganic component, then the polyurethane was soaked in the slurry (mg OMP(OMD)/mg PU = 12) and dried at 60°C. In order to remove the sacrificial polymer and the blinder and to sinter the inorganic components, the impregnated PU was heated at 1300°C. The obtained scaffolds (named OMPs and OMDs) showed a contraction of the dimensions maintaining the porous structure of the plain polyurethane.

They were characterized by XRD and ATR FT-IR and their morphology was investigated by scanning electron microscope (SEM) which showed the presence of merged micrometric or submicrometric particles to form an interconnected and inherent porous structure, in addition to the macro-porosity due to the sacrificial template. In vitro bioactivity properties of OMPs and OMDs were evaluated as well.

The obtained scaffolds were properly loaded with silver nanoparticles to give them antimicrobial activity and then were coated with PLGA for improving mechanical properties and structural integrity and for loading simvastatin, a drug with osteogenic properties [4].

Scaffolds were characterized by XRD, ATR FT-IR, SEM and silver and simvastatin contents Moreover their mechanical properties at the microscale were analyzed by Brillouin microscopy.

Finally in vitro bioactivity, simvastatin and silver release and antimicrobial and antibiofilm activities were evaluated.

Good results were obtained in all the evaluated profiles and thus these scaffolds deserve to be further investigated under other profiles such as cytotoxicity and ability to induce osteogenesis.

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POSTER N. 25

NANO-CARRIERS AS MICROENVIRONMENT REGULATORS FOR TISSUE ENGINEERING STRATEGIES

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Controlled release devices can be extremely relevant for tissue engineering strategies, acting as microenvironment regulators for the sustained delivery of specific biological signals, biomolecules or drugs. For example, an hydrogel matrix casted or bioplotted can be functionalized with poly-lactic-co-glycolic acid nano-particles (PLGA-MCs) carrying different Growth Factor (GFs) payload, to provide a 3D biomimetic environment with the capacity to direct stem cell commitment towards specific phenotype. Alternatively, these controlled delivery system can be loaded with specific drugs to explore in vitro cells effectiveness in recovery their impaired status due to pathological disorder.

The work will illustrate several case studies related to PLGA-nanocarriers obtained with advanced dense gas technologies, loaded with specific growth factors and seeded with human Bone Marrow Mesenchymal Stem Cells (hBM-MSCs) into different hydrogel matrix to promote specific cells committment. Proper release profiles of growth factors were succesfully achieved in all cases, in order to promote stem cells differentiation, as indicated by transcriptional upregulation of costituents genes along the culture, monitored by qRT-PCR. Histological and quantitative-immunofluorescence (qIF) analysis confirmed cell activity in remodeling the synthetic extracellular matrix. Furthermore, static culture of the 3D constructs lacked evidence of specific gene overexpression; whereas, dynamic cultures always showed better performance suggesting that proper growth factor releases from PLGA carriers were promoted only by dynamic culture systems who assured proper mass transfer and exchanges. The described study supports the use controlled delivery nano-carriers for functionalising synthetic extracellular matrix in order to assembly in-vitro 3D biomimeticculture to study regenerative events and patological in vitro model.

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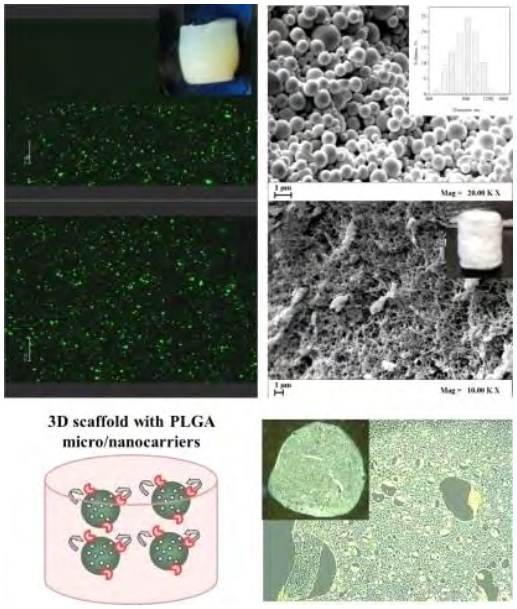

Fig. 1 Examples of 3D bioengineered culture with nanocarriers as micro-environment regulators.

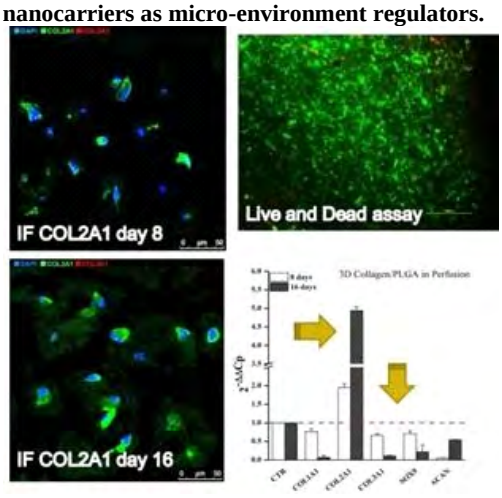

Fig 2. Condrogenic commitment of 3D hBM-MSC culture into collagen scaffold loaded with hTGFβ1 releasing PLGA beads.

POSTER N. 26

ORGAN-RETENTIVE OSMOTICALLY DRIVEN DELIVERY SYSTEMS: FABRICATION OF AN H-SHAPED DEVICE AND CHARACTERIZATION OF THE OSMOTIC UNIT

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Introduction

Growing attention has been drawn by systems suitable for being retained within hollow organs, such as the stomach and urinary bladder [1]. The main goal is to increase the drug dose fraction delivered to the target site, thus improving the outcome of local therapies. Moreover, gastro-retentive delivery systems could be effective in enhancing the bioavailability of drugs having either an absorption window in the upper gastrointestinal tract or poor solubility/stability in the enteric environment [2]. In both cases, advanced formulation strategies and manufacturing technologies would be needed to combine a sufficiently long-lasting retention with prolonged release of the bioactive compound regardless of the patho-physiological variables found at the disease site (pH, ionic strength, volume, composition and hydrodynamics of the medium, presence of enzymes, mucus, *etc.*) [1]. The time course of residence of the delivery system within the target compartment should approximatelycorrespond to that of release of the active ingredient and be as long as possible when it comes to chronic treatments.

While more consolidated strategies are available to reduce the drug release rate, reliable organ retention is still a challenging goal. Different systems for extended residence time have been proposed so far, which are mainly based on bioadhesion, buoyancy and size enlargement. According to the latter approach, an original small-sized configuration is required to make administration possible, followed by attainment of a sufficient spatial encumbrance to prevent early emptying from the organ.

The size enlargement can be based on various physical mechanisms, such as swelling brought about by glass-rubber transition, elastic recovery or inherent shape memory properties of the materials in use. When taking on their bulky configuration, retentive systems are also expected to be stiff enough to resist mechanical forces possibly exerted by the organ and be subject to dissolution, biodegradation or disassembly toshrink and be spontaneously voided from the organwhen exhausted.

On the basis of the above premises, a recently described organ-retentive drug delivery platform leveraging osmotic pressure for increase in size, the Organ-Retentive Osmotically Driven System (ORODS), was herein fabricated in an H-shaped configuration and characterized for size, mechanical properties and osmotic agent concentration over time.

Experimentals

Materials

Paracetamol CD (Rhodadap® DC90, Rhodia Wuxi Pharmaceutical, RPC), hypromellose (HPMC (Methocel® K4M, Colorcon, UK), microcrystalline cellulose (Avicel® PH200, FMC, US), methacrylic adhesive solution, acrylicglue, hard gelatine capsule size 00 (Coni-Snap®, Lonza, BE), Dialysis membrane made of regenerated cellulose having a cutoff of 12-14000 Dalton (Spectrum Labs™ Repligen, Spectra/Por™ 2, USA); acrcylic glue (Loctite, DE).

Methods

Fabrication of the H-shaped device

The prototypes were fabricated by manually inserting a tube made of regenerated cellulose (osmotic unit) into the through holes (elliptical opening of 5.0x1.5 mm) of 2 hydrophilic matrices (500 mg) perforated by a precision driller (Proxxon, DE). The matrices were obtained by tableting (tablet press FA/8, Officine Ronchi, IT) using 20x8 mm concave punches and a compaction force (Fa) of approximately 10 kN, from a powder mixture containing paracetamol as a tracer drug (Table I). The osmotic unit (average diameter and flat width of 3.7 mm and 4.8 mm, respectively) was obtained by foldinga plain membrane of defined area and layout and gluing the edges together. 50 mg of sodium chloride was loaded inside as an osmotic agent. The assembled devices were gently folded and inserted into hard-gelatin capsules.

Table 1. Composition of hydrophilic matrices

Component	Percent amount (%)
Paracetamol	50
Hypromellose Methocel K4M	40
Microcrystalline cellulose, Avicel PH200	10
Total	100

Characterization of the H-shaped device

Isolated osmotic units were immersed in deionized water (800 mL, 37.0 ± 0.5 °C), and their diameter was measured (n=3, 5 different positions) at established time points using a digital caliper (Absolute Digimatic CD-15CP, Mitutoyo, UK). The corresponding volume of filled tubes was th. Mechanical testing of isolated osmotic units (n=3) after 2 h ofimmersion in deionized water was performed adapting the standard test method for flexural properties of unreinforced and reinforced plastics and electrical insulating materials American Standard Testing Material (ASTM) D790 A TA- XT2 analyzer (Texture Technologies, Hamilton, US-MA)

was used equipped with a three-point bend fixture (support span of 22 mm, support cylindrical rods and nose of 2 mm in diameter, 50 N load cell) and software for analyzing displacement and load. The support and nose components of the equipment were purposely designed and fabricated via Fused Deposition Modeling 3D printing from commercially available PLA filament. Release of sodium chloride from osmotic units containing 50 mg of salt was evaluated by placing the samples (n=3) in deionized water (800 mL, 37.0 ± 0.5 °C). At pre-established time points, the conductivity of the acceptor medium was measured potentiometrically, basedon which the amount of sodium chloride released was calculated (VWR International, pHenomenal® CO 3100 L, IT).

Results

Fig. 1 shows an H-shaped osmotically-driven system intended for gastric retention after assemblyand folding for insertion into a hard-gelatin capsule, so as to enable oral administration. When in contact with water, the gelatin capsule dissolved quickly, and the ORODS was set free (Fig. 1A-B). Thus, water inflow started, causing the system to unfold and take on the bulky configuration required for retention (Fig. 1C).

A B C

Fig. 1. H-shaped ORODS in its initial configuration for administration, before (A) and after (B) insertion into a hard-gelatin capsule, and with water-filled osmotic-compartment (C).

The maximum spatial encumbrance, as assessed by measuring the immersed osmotic unit in size over time, was reached within 2 h (Fig. 2). However, the units began to enlarge right away, and approximately 80% of maximum volume was achieved in the first 30 min.

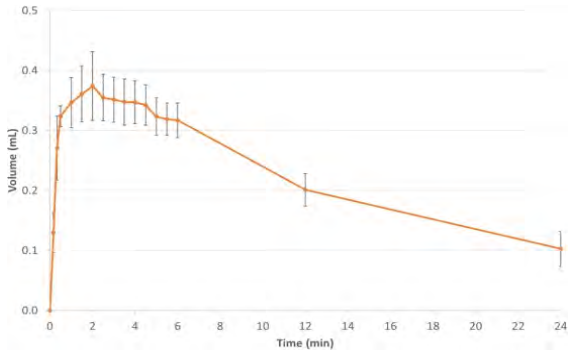

Fig. 2. Mean volume *vs*. time profile for isolated osmotic units of H-shaped ORODS immersed in deionized water (n=3, vertical bars represent standard deviation).

Gradual shrinking occurred after about 6 h, and at 24 h the volume was reversed to 20% of maximum. As the volume increased, the osmotic unit acquired stiffness. The mechanical strength developed by an isolated osmotic unit over time following water inflow was assessed approximately at the time at which the maximum expansion was achieved (Fig. 3). The downward displacement of the nose of the three-point bend fixture caused an increase in the force opposed. The highest average value recorded in triplicate was 136 g ± 8 sd.

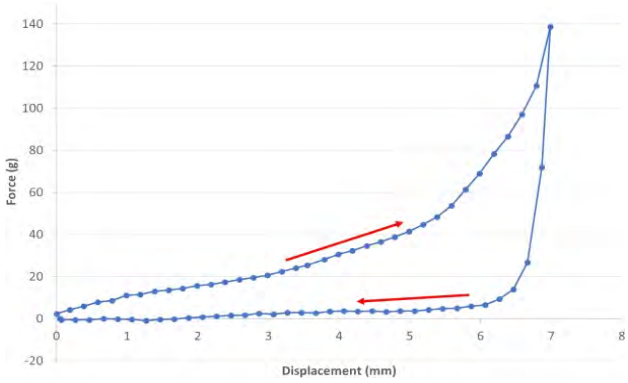

Fig. 3. Force *vs*. displacement profile obtained from the isolated osmotic unit of an H-shaped ORODS at its maximum volume (2 h immersion in deionized water).

The loss of sodium chloride from the osmotic unit was also evaluated by measuring the salt concentration in the receptor medium. As shown in Fig. 4, the calculated amount of sodium chloride in the osmotic unit decreased rapidly. The time course of salt content and of volume of the unit were not aligned. However, as demonstrated by the mechanical test, the residual sodium chloride levels were sufficient to ensure that adequate stiffness was maintained.

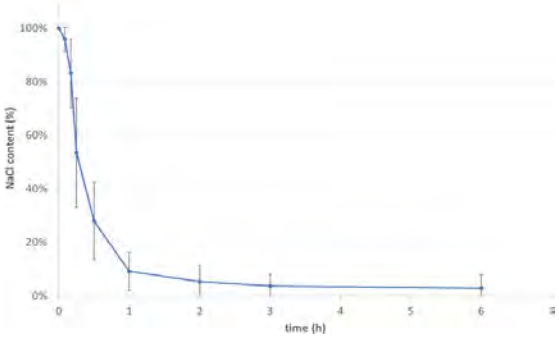

Fig. 4. Mean residual amount of sodium chloride in isolated osmotic units of H-shaped ORODS immersed in deionized water (n=3, vertical bars represent standard deviation).

Conclusions

An H-shaped system was fabricated and evaluated according to a recently proposed technology for osmotically driven organ-retention and delivery. The results obtained in terms of changes in volume and stiffness undergone by the isolated osmoticcompartment turned out potentially suitable for the pursued *in vivo* performance.

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POSTER N. 27

MAGNETIC SCAFFOLDS FOR THE MECHANOTRANSDUCTION STIMULATION IN TENDON TISSUE REGENERATION

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Tendon pathologies are medical conditions that include ruptures and overuse injuries accompanied by inflammatory and degenerative alterations, such as tendinopathies and tendinitis. Current clinical grafts used in tendon surgery require long periods of treatment, often resulting in tissue re-rupture. For this reason, innovative strategies need to be explored. Scaffolds represent an attractive three-dimensional substrate for tendon regeneration, as they offer structural support for cell organization and stimulate the reconstitution of the native extracellular matrix. Moreover, the use of magnetite nanoparticles has been gaining a lot of interest in recent years due to their capability to control cell signaling both in vitro and in vivo by mechano-stimulation. The nano-movement induced by the magnetic field on the scaffolds seems able to cause forces in the range of pN, and cells act in response to those mechanical stimuli allowing deeper tissue reparation [1]. Given these premises, the aim of the present work was the design and the developmentof fibrous scaffolds based on polyhydroxybutyrate (PHB), a thermoplastic biodegradable polymer, doped with magnetic iron oxide nanoparticles (Fe_3O_4) and coated with gelatin (Gel), able to mimic the hierarchical structure of the tendon and to improve the tissue healing potential.

Experimental methods.

PHB was solubilized in acetic acid, and Gel and Fe_3O_4 were added. The blends were spun using a centrifugal spinning apparatus and the obtained fibers (namely PHB, PHB-Fe_3O_4, and PHB-Fe_3O_4-Gel) were collected in order to form scaffolds with an aligned orientation. The systems morphology and the incorporation of magnetite were assessed using SEM and SEM-EDX and the surface wettability was evaluated by means of contact angle measurements.

The superparamagnetic behavior of the scaffolds and their mechanical properties were tested. Moreover, their weight loss in physiological medium was evaluated. Finally, cell adhesion and proliferation in vitro were assessed on fibroblasts with and without the application of static magnetic fields of different extent (47mT±1, 155mT±5, and 285mT±5) for 21 days ofculture.

Results and discussion.

The systems are characterized by an aligned structure that could mimic the tendon hierarchical structure, and SEM-EDX shows that Fe_3O_4 nanoparticles are successfully incorporated into the structure. The presence of magnetite increases the scaffolds' rigidity, probably due to the nanoparticles' distribution into the fibrous matrix [2]. Moreover, the presence of Gel leads to a higher fiber swelling and surface wettability.

The scaffolds with magnetite are characterized by a superparamagnetic behavior, fundamental to allow the cell mechanostimulation. In fact, when a magnetic field is applied, the scaffolds could respond with vibrations generating a transient physical force that could be transferred to the host cells [3]. Furthermore, the magnetic measurements allow to calculate the effective % of magnetite loaded into the structure, which results similar to the theoretical one. The scaffolds also show a progressive weight loss in physiological medium, demonstrating a degradation capability while maintaining their morphology and alignment. Finally, the scaffolds with Gel are characterized by a cell growth higher than that of the positive control (cells grown in standard conditions). Interestingly, the application of the magnetic fields also leads to a significant increase in cell adhesion and proliferation onto the systems loaded with Fe_3O_4, reaching values considerably higher than those of the control after 21 days of culture. Moreover, the combination of the scaffolds enriched with magnetite together with the application of the magnetic fields leads to a significant cell alignment, mimicking the tendon fascicles.

Conclusions.

In conclusion, centrifugal spinning was successfully used to prepare scaffolds based on PHB in association with Gel and Fe_3O_4. The developed scaffolds combined with the application of a magnetic field are able to enhance the cell adhesion, alignment and proliferation in vitro, resulting interesting tools for the cells mechano-stimulation. Further investigations will be performed in vivo in order to evaluate the scaffolds regenerative capacity.

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POSTER N. 28

THERMOSENSITIVE HYDROGELS ENRICHED WITH LACTIC BACTERIA FOR VETERINARY APPLICATIONS

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A recent study on the developments of thedairy market in the world evidenced that in 2020 milk production reached 906 million tons, of which 236 million only in Europe [1]. The development of automated farms is the answer to the growing milk demand to increase production, even if this solution has caused reduced fertility of dairy cows, resulting from stress [2]. Moreover, in the *post-partum* period, the immune system lowering and vaginal dysbiosis can lead to vaginal and uterine infections, compromising the health and fertility of dairy cows [3]. The most common approach to treat uterine infections in dairy cows is administering antibiotics; however, to oppose antibiotic resistance, efforts are being made to limit their use as much as possible.

This research work aimed to formulate an intravaginal gel enriched extemporaneously with probiotics for the preventive treatment of *post-partum* uterine infections in dairy cows. These gels had to satisfy specific requirements; in particular, they must be thermosensitive to the animal's body temperature (39 °C) to allow good handling by the operator and ease of administration. At room temperature, they ought to be liquid (sol-phase), and they would undergo gel transition after administration, allowing the spreading and coating of the vaginal mucosa.

Poloxamers 407 (P407) and 188 (P188) were chosen as the main components of the gels, and the study included a preliminary phase in which, using the experimental design approach, several formulations were examined in order to identify, based on their gelation temperature, those with characteristics compatible with their use. Moreover, to increase the mucoadhesive properties of the thermosensitive gels, the formulations characterized by adequate gelation temperatures have been added with a small percentage of low molecular weight chitosan. All the preparations (with and without chitosan) were completely characterized, evaluating gelation time, pH, viscosity, rheological behaviour, injectability, erosion rate, and strength resistance.

The obtained results demonstrated that the gelation temperature surged by increasing the P188 concentration and was inversely dependent on the total solids concentration. The right balance between these two factors is mandatory to have available suitable preparations. Moreover, the presence of chitosan slightly reduced the gelation temperature compared to that of the corresponding chitosan-free preparation.

The injectability of the preparations in the sol-phase was appropriate. The sol-gel transition was quite rapid (less than 2 min), sufficient to avoid both the spontaneous expulsion of the preparation afteradministration and its premature gelation during theadministration step. The pH of each formulation was compatible with that of the intravaginal tract of the cows, even if those enriched with chitosan were slightly acidic. The viscosity of the formulations was determined at three different temperatures (10, 20, and 28 °C). As expected, it generally raised with temperature, except for one of the chitosan formulations, which did not show a variation of this parameter, regardless of the temperature at which it was determined. This stability represents an important advantage for its practical management and handling in farms.

The *in vitro* erosion of hydrogels was monitored by their weight changes in phosphate buffer solution at pH 6.8. Hydrogels gradually degraded after contact with the fluid, and five hours after the beginning of the test, the percentage of residual gel was over 40% for only two formulations.

The formulation composed of 20.0% P407, 9.5% P188, 0.5% chitosan, and 70.0% acetic acid solution, able to gelify at 35 °C in less than 2 min and characterized by low viscosity at room temperature, was selected as the preparation to be enriched with lactic bacteria. Probiotics could disperse homogeneously into the sol-phase of the preparation, and their presence did not affect the technological characteristics of the gel.

This hydrogel could be suitable for the vaginal administration of lactobacilli in dairy cows, helping to reduce the incidence of *post-partum* vaginal infections; further studies are ongoing to evaluate its effective mucoadhesive attitude and the probiotic viability after dispersion.

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POSTER N. 29

ROS-SENSITIVE MATERIALS BASED ON POLYLACTIC CO-GLYCOLIC ACID FOR CANCER

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Colon cancer is the most frequently diagnosed cancer among the various cancers that normally affect humans on a global scale [1]. Chemotherapy is still mostly administered parenterally, which is a negative factor in terms of patient comfort; therefore, oral administration would be desirable especially for chronic patients requiring long-term treatment [2,3].
From this point of view, polymeric nanoparticles (NPs) are promising for the development of an oral formulation for colon cancer as they protect the drug from various destructive effects of GIT and allow higher local concentrations to be delivered into the colon [4].
The aim of this work was to design, realize and evaluate the performance of polimeric nanoparticles based on PLGA alone or PLGA esterifiedwith 2,2'-[propane-2,2-diylbis (thio)] diacetic acid, loaded with docetaxel (DCX) and docosahexaenoic acid (DHA), as innovative site-specific therapeutic carriers. The technique used to realize nanoparticles was the nanoprecipitation illustrated in the Figure 1.

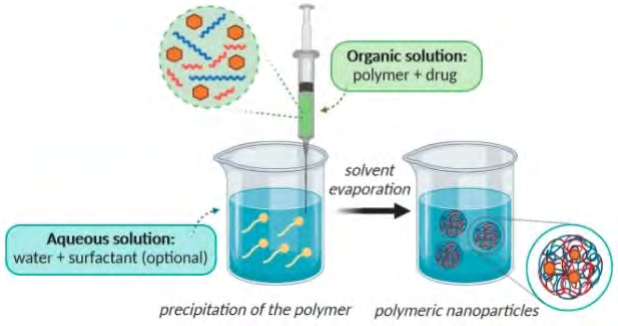

FIGURE 1. Nanoprecipitation technique.

The obtained materials were characterized by FT-IR and ^{1}H-NMR, while dimensional analysis of the nanoparticles obtained was performed by Dynamic Light Scattering and encapsulation efficiency was evaluated. *In vitro* skin permeation tests and antitumor activity on HCT116 cell lines were also conducted. The obtained results showed that the encapsulated drugs are released from the NPs in percentages ranging from 30/35% to 80%, for PLGA-based NPs containing docetaxel and both docetaxel and DHA (Figure 2).

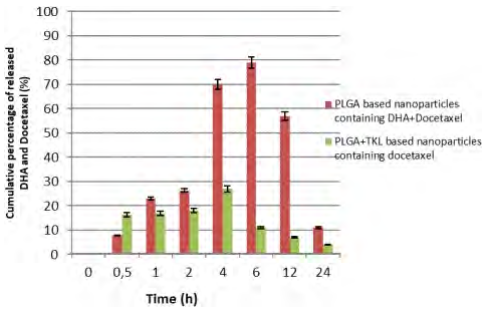

Figure 3. Graph of cumulative release of DHA and docetaxel.

In addition, testing for antitumour activity showed a significant inhibition of cell viability both when the drug was administered alone and in the presence of DHA. The use of this nanocarriers could facilitate the stable and efficient delivery of DCX and DHA through the upper segments of the gastrointestinal tract to the colon. In addition, the presence of the ROS-sensitive 2,2'-[propane-2,2-diylbis (thio)] diacetic acid in their matrix should promote the site-specific release of DCX and DHA in the cancer pathology zone, where high levels of reactive oxygen species could be found.

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POSTER N. 30

EMULSIFICATION AND SPRAY DRYING TO OBTAIN MICROCAPSULES AS CARRIERS FOR LIPOPHILIC COMPOUNDS

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Lipophilic compounds can represent a bioavailability challenge because of their low solubility in water and, consequently, in physiological fluids. Moreover, they could be susceptible to degradation when exposed to light, temperature, and/or oxygen.
Oil-in-water (O/W) emulsions are currently used for lipophilic bioactive substance administration, increasing their bioavailability, but these dispersed systems frequently may be not so stable for a long time [1]. In this context, spray drying (a reproducible, and affordable process) may represent a practical solution to convert O/W emulsions into solid stable microcapsules presenting a heterogeneous polynucleate structure: lipophilic compartments are embedded in a hydrophilic matrix [2]. The present work focused on the formulation of O/W emulsions with adequate characteristics (mainly stability and viscosity) to be processed by spray drying obtaining polynucleate microcapsules: a hydrophilic polymer in the external phase is the support for the lipidic droplets.
The experimental plan involved a preliminary and an optimization phase. The first one started with the preparation of O/W emulsions: they were similar as regards the final content of vegetable oil mixture (7% w/w) and maltodextrin (20% w/w) but differed for the type of natural emulsifier tested (0.5% w/w): soy protein (E1), rice starch (E2), pea protein hydrolysate (E3), carrageenan λ (E4). The two phases wereemulsified by Ultra-Turrax® (16,000 rpm) for five minutes. At different time intervals (0, 15 min, 30 min, 1 h, 2 h), the appearance of the obtained emulsions and their physical stability were evaluated to select the most adequate to be treated by spray drying. E3 and E4had the oily phase well dispersed, with E3 less stable than E4, so the latter was submitted to spray drying treatment. The spray drying process was carried out with an inlet temperature (T_{in}) of 130 °C, outlet temperature (T_{out}) of 80 °C (a target outlet temperature to limit eventual product degradation), 100% of aspiration, 1.75 bar of gas atomization and 7% of the pump (3.55 g/min). The process yield was over 70%, but much oil was lost on the tower walls. Moreover,the amount of oil loaded into microparticles (recovered by a liquid-liquid extraction) didn't exceed 30% of the expected. These results demonstrated that maltodextrin alone was not able to display good carrier behaviour, thus, the optimization of the starting emulsion formulations occurred.
Among the emulsions tested in the preliminary step, E1 and E2 were discarded because unstable and E4 was not considered because it was preferred to improve the formulation of a starting system with a lower viscosity (E4 viscosity = 72 cP, 35 rpm, 25 °C). For these reasons, E3 (viscosity of 8.48 cP and acceptable stability) was chosen to be added of arabic gum asemulsifier and film-forming of the lipophilic compartment [3]. The optimized emulsion (coded hereafter E5), composed of maltodextrin (21%), arabic gum (9%), pea protein hydrolysate (1%), oil vegetable mixture (7%) and water, had a viscosity of 73 cP (35 rpm, 25 °C) and was stable for more than 24 hours, with a homogenous dispersion of oil droplets, d90 = 4.944 μm. Finally, E5 emulsion was easily processed by spray drying.
Four microparticle batches (T1, T2, T3, T4) were produced changing spray drying process parameters. Process yield (PY) and oil recovery (OR) from final microparticles, residual humidity, particle size distribution and flowability of the collected powders were evaluated.
T4 was the most promising powder with a PY of about 88% and OR of over 89%. The residual humidity didn't exceed 7%; microparticles had discreet flowability and a d90 of 42.74 μm.
In conclusion, a polynucleate microcapsule formulation has been optimized by emulsification and spray drying: lipophilic compartments have been embedded in a micrometrics hydrophilic matrix and will be loaded with sensitive lipophilic drugs.

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POSTER N. 31

DESIGN OF A NEW BIOMATERIAL BASED ON HYDROLYZED COLLAGEN AND CHITOSAN AND ITS USE IN THE DEVELOPMENT OF FILMS AND MICROPARTICULATE SYSTEMS FOR TISSUE REGENERATION

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Chronic diseases, congenital disorders and infections could lead to life-threatening situations, followed by degeneration or malfunction of living cells, tissues and organs. In addition to these reasons, natural disasters, accidents and wars also lead to the need to develop new treatments that can promote the regeneration of the anatomical structures involved.

The main aim of the present work was the development of scaffolds with different geometries based on a new biomaterial resulting from the interaction between chitosan (Cs) and hydrolysed collagen (HC). In particular, the proposed systems are films and microparticles, intended for tissue repair and regeneration purpose. Moreover, the loading of ascorbic acid (AA) as model molecule, in both films and microparticles, was investigated.

Firstly, HC (Kelisema, I) aqueous solutions were prepared at different concentrations (0.5%, 1%, 2% w/w) and mixed at 1:1 ratio with a 1% w/w medium MW Cs (Sigma Aldrich, I) solution. The resulting mixtures were characterized in terms of conductivity, pH, viscosity and turbidimetry.

Such mixtures were then used for the production of film by solvent casting and microparticles by spray drying. Regarding films, in order to achieve their insolubility in aqueous medium; pH of the mixtures (pH 3.5) was increased up to three different pH values (4.5, 5 and 5.5) insoluble films were successfully obtained. As for microparticles pH modification was not necessary since they were already insoluble in aqueous medium probably due to the spray drying process.

Films were characterized for: i) physicochemical properties by FT-IR measurements; ii) mechanical properties by tensile technique; iii) wettability by contact angle measurements. Films were also subjected to in vitro biodegradation studies. As for mechanical properties, films demonstrated to be characterized by different stress-strain profiles depending on the pH of the starting mixture. In fact, films obtained from mixtures at pH 3.5 showed a typical behaviour of brittle material, indicating that such films are able to withstand high tensile forces without the ability to deform plastically. Instead, in the case of pH-modified films (pH 4.5), stress-strain profiles typical of plastic materials were observed. Moreover, the results of biodegradation studies indicated that films had a different behaviour depending on HC concentration. In particular, an increase in the degradation rate was observed on increasing HC concentration.

Microparticles were characterized for: i) morphology and size by SEM analysis; ii) stability by evaluating zeta potential; iii) physicochemical properties by TGA, DSC and FT-IR techniques.

Microparticles independently of HC concentration are characterized by a mean diameter of 5 μm and by a positive zeta potential (>30 mV) that was stable for at least 7 days.

Moreover, the addition of AA was considered for both films and microparticles. AA-loaded films and microparticles were successfully achieved and characterized by morphology studies and FT-IR and thermal analyses. The AA release from both films and microparticles was evaluated by spectrophotometric measurements. Both the samples proved to be able to allow a prolonged release of the model molecule loaded, reaching % released values under 40% after 24h.

Finally, all the formulation developed were subjected to in vitro biocompatibility tests towards three different cell line models: normal human dermal fibroblasts, Schwann cells (murine) and macrophages (murine). As a result, all samples showed high biocompatibility when put in contact with the three cell lines employed.

In conclusion, the complex based on HC-Cs complexes proved to be a promising biomaterial for the manufacturing of scaffolds for tissue regeneration.

In particular, biocompatible and biodegradable films and microparticles able to prolong the release of a loaded model drug were obtained.

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POSTER N. 32

HYALURONAN-ESTRADIOL NANOGELS AS POTENTIAL DRUG CARRIERS TO TARGET ER+ BREAST CANCER CELL LINE

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The delivery of bioactive molecules to the target site has attracted increasing attention over the past three decades as a turning point in the treatment of several diseases [1]. In this respect, polysaccharide-based nanosystems able to control and target the delivery of useful molecules turned out to be the very promising [2]. Polysaccharide functionalization with small hydrophobic molecules allows to obtain amphiphilic derivatives, which can spontaneously self-assemble in water resulting in the formation ofnanogels (NHs). These nanosystems are highly versatile as they can encapsulate both hydrophilic and hydrophobic drugs [3]. Moreover, they offer benefits interms of rapid drug release rates and good targeting ability through the easy functionalization of the polymeric backbones [4]. In fact, grafting the polysaccharide with a specific ligand could enhance the NHs selectivity towards cells that have receptors capable of selectively recognizing it [5].

In this light, an innovative hyaluronan-based nano-delivery system is proposed for the active targeting towards ER+ breast cancer. Hyaluronic acid (HA), an endogenous and bioactive anionic polysaccharide, was functionalized with estradiol (ES), a sexual hormone involved in the development of some hormone-dependent tumors, to give amphiphilic derivatives (HA-ES) with theoretical derivatization degrees (DD)in a range from 5% to 70% mol/mol.
Derivatives were dispersed in water and autoclaved at 121 °C for 20 min to assess those able to self-assemble in water. The obtained ES-NHs were characterized by DLS, and the ones prepared with a DD of 40% were selected as the best formulation.
ES-NHs ability to entrap hydrophobic molecules was also investigated by loading curcumin (CUR) and docetaxel (DTX), both able to inhibit the growth of ER+ breast cancer, following two different strategies. CUR was loaded during NHs formation while DTX was loaded in a double-step procedure, after the autoclaving process. The obtained formulations showed a small hydrodynamic diameter, low PDI and ahigh ζ-potential value. The encapsulation efficiency and drug loading were calculated, resulting in a solubility enhancement of 70x and 7x, respectively.

Furthermore, the formulations were studied for their capability to inhibit the growth of the MCF-7 cell line, showing that DTX/ES-NHs ability to inhibit cell proliferation was higher than that of free DTX.

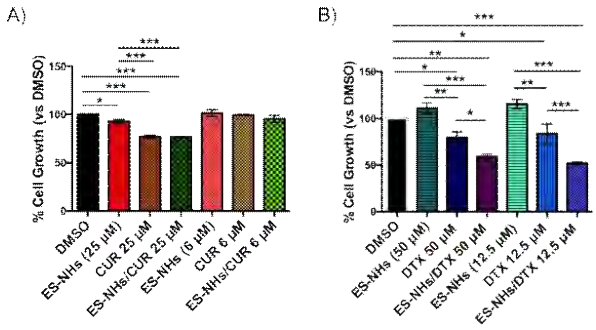

Fig.1 A) Effect of ES-NHs/CUR, ES-NHs and CUR and B) of ES-NHs/DTX, ES-NHs and DTX on MCF-7.

Finally, internalization assays were performed to follow the cell distribution of the nanosystems. In vitro uptake experiments showed that, after 30 minutes of incubation, fluorescent-labelled ES-NHs were in vesicle-like structures, partly near cell membrane and partly near the nuclei, suggesting a rapid intracellular uptake and assuming a receptor-dependent targeting.

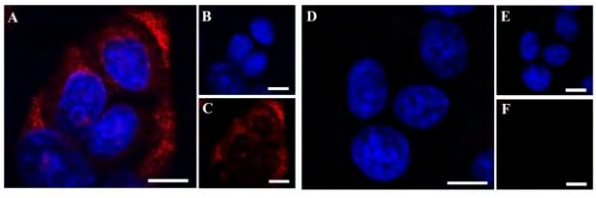

Fig.2 Fluorescence analysis of MCF-7 cells incubated with fluorescent ES-NHs for 30 min: A) merge, B) DAPI, C) ES-NHs; D) merge, E) DAPI, F) free Rhod.

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POSTER N. 33

DESIGN OF DEFORMABLE VESICLES BY PROLIPOSOMAL APPROACH: STRUCTURAL AND SKIN PENETRATION PROPERTIES

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Introduction

Deformable liposomes represent valuable drug carriers for cutaneous administration. Nevertheless, the fluid lipid membrane can favour the drug leakage during storage. Proliposomes may represent a suitable strategy to solve this issue. As an alternative, a novel carrier, which encloses hydrophobic drugs in the inner core of vesicles, namely a drug-in-micelles-in-liposome system (DiMiL), has been proposed[1]. In this work, we investigated the possible advantages of combining these two approaches to obtain a formulation able to enhance the skin penetration of cannabidiol (CBD).

Methods

Preparation of proliposomes

Proliposomes were composed of soy-phosphatidylcholine (s-PC) and Tween® 80 (T80) in weight ratio of 85:15; trehalose, sucrose or lactose were added in 1:1 or 1:3 sugar:lipid weight ratio. Powder formulations were obtained by the following methods:

Spray-drying (SD) – The feeds were prepared by suspending the sugar in the lipid organic solutions (ethanol and methanol were tested as solvents) or dissolving the sugar and the lipid components in water/organic solvent mixtures. Different concentrations of the components were screened. Feeds were spray-dried by a 4M8 spray-drier (Procept, Netherlands), equipped with a 0.6 mm nozzle. After a preliminary evaluation, the inlet temperature and the feed flux were set at 150 °C and 10 mL/min, respectively.

Slurry method (SL) – For the preparation of proliposomes, s-PC and T80 were dissolved in ethyl acetate in a round-bottom flask. Sugars, previously micronized, were added. The solvent was mostly evaporated at 40 °C under reduced pressure by using a rotavapor and then the slurry was completely dried at 70 °C overnight. The resulting dry powder was uniformed by sieving the powder through a 355-µm sieve.

Proliposomal powders were characterized in terms of granulometry, water content and flowability. Oxidation of lipids was also studied after the drying process.

Preparation of DiMiL formulations

DiMiL formulation were obtained by hydrating proliposomal powders with a 10% w/v Kolliphor micellar dispersion, containing 3.5 mg/mL CBD when appropriate. Particle size was uniformed by 5 min sonication (30 sec on and 30 sec off). As control, DiMiL systems were also prepared by the conventionalthin lipid film hydration method (FH-formulations, Table 1). DiMiL were purified by size exclusion chromatography. DiMiL systems were characterized in terms of particle size distribution and ζ-potential (by DLS), encapsulation efficiency (EE%) and deformability (using a dynamometer assisted extrusion assay)[2].

The structure and morphology of the vesicles was investigated by small-angle X-ray scattering (SAXS) and cryo-EM.

The in vitro drug release and in vitro skin permeability assays were carried out using Franz diffusion cells method and using nitrate cellulose and human epidermis as a membrane, respectively.

Results and discussion

Proliposomal powder

Spray-drying. The use of co-solutions was preferred to the suspensions due to issues related to the clogging of the tubing and nozzle. In particular, the use of sucrose and a methanol:water solution in 3.4:1 ratio allowedthe preparation of physically stable feeds with a total solid content of 12 % w/v. The process yield increased with the sucrose content because the carrier limited the lipid adherence to the glass wall of the drying chamber. Indeed, the yield was improved from about 60% to about 90% for s-PC:sucrose ratio 1:1 and 1:3, respectively. Proliposomes had a diameter smaller than 10 µm.

Slurry method. Mixtures at the lowest s-PC:sugar ratio led to wax-like and unprocessable materials. Moreover, only trehalose provided suitable powders with a mean diameter of about 330 µm.

Both drying methods did not cause phospholipid oxidation since no significant variations of the oxidation index were observed with respect to raw s- PC.

Preparation and physico-chemical characterization of DiMiL systems

The hydration of proliposomes with micelle dispersion allowed to obtain a monodisperse population of liposomes with the desired particle size (**Table 1**). The

presence of the sugar in the aqueous core of liposomes, not removable by purification, did not affected the EE% of CBD and it was comparable to that obtained in liposomes prepared by conventional thin lipid film hydration method (data not shown).

Table 1-Composition and main characteristics ofDiMiL systems

Form. code	sPC/ sugar ratio	D (nm)	PdI	K (mN/mm)
S1	1/1	118	0.4	10±2
S1.5	1/1.5	109	0.3	10±3
S2	1/2	108	0.3	10±2
S3	1/3	117	0.3	13±3
S2-C	1/2	95	0.3	1±0
T2	1/2	112	0.3	11±4
T3	1/3	129	0.3	9±2
T2-C	1/2	129	0.3	4±3
FH0	--	117	0.1	8±1
FH10	--	125	0.1	7±3
FH0-C	--	106	0.1	3±0
FH10-C	--	140	0.2	2±0

Note: S: sucrose (SD method); T: trehalose (SL method); C: CBD. Micelle Content: 10%, w/v for S and T series. FH: liposomes prepared by thin lipid film hydration method; FH10: DiMiL at 10%, micelle concentration. FH0: control deformable liposomes without micelles. The st.dev. on D is lower than 1 nm, on the PDI is lower than 0.05.

All formulations resulted deformable even if the CBD caused a slightly decrease of the *K* values. This variation could be attributed to a partial fusion of CBD carrying micelles into the external bilayer and to a fludizing effect of CBD. In fact, thermal behavior of a model membrane made of 1,2-dipalmitoyl-sn-glycero- 3-phosphocholine (DPPC) significantly changed in presence of CBD since after drug encapsulation themain transition temperature of DPPC shifted from 42.2±0.1 °C to 37.3±0.5 °C with a concomitant reduction of the transition enthalpy (from 37.0±0.5 to 16.3±1.0 J/g.). Basing on the quality attributes of both proliposomes and final DiMiL dispersions system with 2:1 sugar/lipid ratio were selected for further studies.

Structural analysis of DiMiL systems

Cryo-EM images clearly showed the micelles in liposomes structure of DiMiL. Micelles were uniformly present in the core of analyzed DiMil systems (**Figure 1**). No trace of micelles was revealed in the dispersing medium suggesting the stability of the formed system after preparation.

SAXS data were in agreement with cryo-EM but evidenced also a partial repartition of micelles in the bilayer. Nevertheless, the number of kolliphor micelles found in the core of DiMiL was very close to the theoretical value.

The encapsulation of CBD in the systems did not affect their key structural properties, as well as the presenceof sugars.

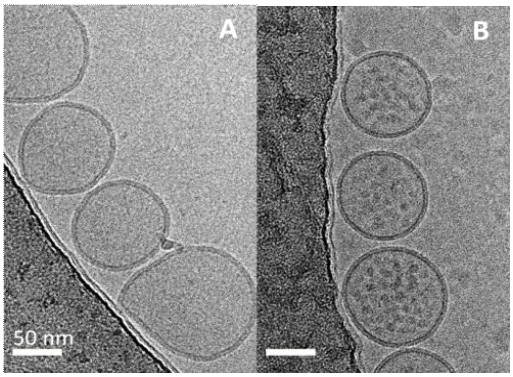

Figure 1. Cryo-EM of (a) conventional deformable liposomes, FH0; (b) DiMiL system.

In vitro drug release

The release rate constants of DiMiL formulations were significantly lower ($p<0.05$) with respect to that of conventional deformable liposomes, regardless of the preparation method, confirming that the encapsulation of CBD loaded micelles in the inner core of the liposomes assures a better control of the drug release.

In vitro skin permeability studies

The encapsulation of the drug in the DiMiL system improved the in vitro skin permeation of CBD with respect to results obtained using a solution of CBD in vaseline oil and conventional deformable liposomes;the CBD permeation flux was increased about 5 and 3 times, respectively. Furthermore, the lag time was significantly shortened since FH10-C and S2-C allowed the detection and quantification of the drug in the receiver solution already after 3 and 5 h, respectively. The presence of residual trehalose in the formulation T2-C resulted in a significant improvement of CBD permeation with respect to FH10-C and S2-C. Lastly, despite the strong affinity of CBD for stratum corneum lipids, the amount found in the epidermis at the end of the in vitro permeability studies after application of DiMiL systems was almost 10-fold lower than those measured using vaseline oil. DiMiL istherefore confirmed to be a drug carrier for the skin delivery of poorly permeable compounds.

Conclusions

The obtained results demonstrated that proliposomes may be a valuable intermediate for the preparation of deformable liposomes based cutaneous dosage forms, able to control the release and improve the permeation of CBD.

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POSTER N. 34

LIGHT UP A FIRE INSIDE THE TUMOR BY COMBINING NEXT GENERATION ONCOLYTIC VIRUSES WITH EXTRACELLULAR VESICLES

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Cancer cells employ sophisticated mechanisms to evade and suppress the immune- recognition and, consequently, the anti-cancer immune responses thus generating a "cold" immunosuppressive tumour microenvironment to avoid the immune attack [1]. In this scenario oncolytic viruses (OVs), which are vectors able to kill cancer cells spearing the healthy ones specifically, represent a new dawn to convert tumour immunosuppression to immunomodulation and improve the efficacy of cancer treatment. Oncolytic viruses display unique anti-cancer mechanism compared to conventional therapies, allowing the possibility for synergistic anti-cancer effect [2]. Wehave hypothesized that by combining a newly cloned oncolytic virus expressing potent co-stimulatory molecules such as: CD40L and ICOS ligands with anti-PD-1 inhibitors we could enhance anti-cancer efficacy by efficient priming of anti-tumour immunity. Emerging preclinical and clinical findings confirm that OVs can modulate anti-cancer immune responses that enhance the efficacy of checkpoint inhibitors. Therefore, the combination therapies of oncolytic vectors with check-point inhibitors are an encouraging regime for cancer treatment by lighting up a fire inside the tumour, thus creating a pro-inflammatory tumor microenvironment. We reported that the administrationof a novel oncolytic adenovirus Ad5/3-D24-ICOSL- CD40L induced the production of tumour infiltrating lymphocytes to the site of injection. Furthermore, in immunocompetent melanoma and mesothelioma mouse models, we first showed that the therapy withthe OVs in combination with anti-PD1 was the most effective regimen in both tested animal models then we evaluated the possible correlation between tumour infiltrates and anti-cancer efficacy. Described results showed that the delivery of Ad5/3-D24-ICOSL-CD40L in combination with anti-PD1 resulted in *in vivo* synergistic inhibition of both melanoma and mesothelioma tumours in mice with established AB12 and B16V tumors. Importantly anti-cancer effect positively correlated with cytotoxic CD8+ tumour- infiltrating lymphocytes exerting a central role in the tumour volume control thus generating beneficial outcomes that will showcase the possibility of using this approach to combat cancer. Furthermore, the possibility of using extracellular vesicles, which are cargo delivery vehicles for the systemic delivery would extend the OV application to the treatment of metastatic tumors.We initially showed Therefore, by using *in vivo* and *ex vivo* imaging technologies, as a detection system for the characterization of the whole- body biodistribution of EV-formulations, we demonstrated a selective delivery of cancer-derived EVs to the neoplastic tissues along with the induction of tumor-associated inflammatory reactions, which are characterized by immunogenic cell death and CD3+/ CD4+/CD8+ T-cell infiltration. Altogether, our findings strongly support the systemic administration of anticancer agents encapsulated into Evs thus offering a glimmer of light to cancer patients.

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POSTER N. 35

FORMULATION OF STEALTH CATIONIC LIPOSOMES FOR THE DELIVERY OF PLASMID DNA

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Gene therapy has emerged as a remarkableand promising therapeutic option with immense potential for various biomedical applications, such as gene editing, vaccines and immunotherapies [1]. Gene delivery is one of the great challenges of this field and the development of an efficient delivery platform can really change the outcomes of gene therapy [2]. Negatively charged genetic material has to face the crossing of cell membranes and is susceptible to rapid degradation by nucleases or in lysosomes. Cationic liposomes have shown promise as potential carriers for gene delivery, as they offer protection to the genetic material (pDNA), facilitate transport, enhance internalization, and enable targeted release at the desired site of action.

In this study, we prepared stealth cationic liposomes using microfluidic techniques. Microfluidics is an effective method for the production of liposomes in reproducible batches with highly controlled and homogeneous particle size [3]. The liposomes were formulated employing cationic lipids (52 mol%) with a permanent positive charge (DOTAP) or ionizable(DLin-KC2-DMA), which allow the complexation of plasmid DNA. The cationic lipids have a crucial role on the encapsulation efficiency of the plasmid DNA as well as on its release. The formulations also included cholesterol (38.5 mol%) for stability and HSPC (8 mol%) as a helper lipid. Lipid components were dissolved in ethanol at a concentration of 10 mM in terms of total lipid. Additionally, PEG-DSPE (1.5 mol%) of 2kDa was incorporated to create a hydrophilic shield, preventing vesicle aggregation, evading opsonization, and slowing clearance by the RES (reticuloendothelial system).

Initially, we investigated the impact of process parameters on particle size and polydispersity indexes by varying total flow rates (TFR) and flow rate ratios (FRR) between the organic and aqueous phases. Subsequently, liposomes were prepared using different ratios of lipid positive charges to DNA negative charges (N/P ratio of 2, 4, 6, 8, 10) through directcomplexation of pDNA during liposome formation. The aqueous phase was prepared by diluting pDNA in 25 mM sodium acetate buffer, pH 4. The liposomes were characterized using dynamic light scattering (DLS) for size, PDI, and zeta potential. The morphology was evaluated by TEM and the plasmid cargo was analyzed by agarose gel. Encapsulation efficiency was determined through PicoGreen assay.

Under the selected process conditions (TFR 8 ml/min, FRR 3:1), DOTAP formulations maintained a constant size (86-98 nm) and PDI (0.23-0.27) from N/P 4 to 10, except for N/P 2, which exhibited increased size (130 nm) and PDI (0.36). For DLin-KC2-DMA formulations, size increased as N/P ratio decreased (from 77 nm for N/P 10 to 206 nm for N/P 2), while PDI remained below 0.2. The zeta potential rangedfrom +20 to +30 mV for DOTAP liposomes, while DLin-KC2-DMA vesicles had a zeta potential of -10 mV. All liposomes have a comparable spherical morphology. Encapsulation efficiency was consistent (96-98% for DOTAP and 77-80% for DLin-KC2-DMA liposomes) from N/P 4 to 10 but decreased for N/P 2.

Finally, we investigated the transfection efficiency of the encapsulated pDNA, encoding for the TdTomato fluorescent protein. The liposomes were incubated with myogenic cells for 24h and the fluorescence was observed after 36h. DLin-KC2-DMA liposomes demonstrated superior transfection capacity compared to DOTAP liposomes, particularly at N/P ratios of 6, 8, and 10. The ionizable lipid DLin-KC2-DMA enabled the production of more homogeneous liposomes with sizes below 100 nm (N/P 6, 8, 10), suitable surface charge for in vivo applications, and improved in vitro transfection efficiency compared to liposomes formulated with permanently positive charge lipids.

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POSTER N. 36

ALIMENTARY PROTEIN ISOLATE AND CHITOSAN DERIVATES INTERCATION PRODUCTS AS INNOVATIVE BIOMATERIALS FOR TISSUE ENGINEERING

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Polysaccharides (PL) and proteins (PR) are among the most widely used biopolymers in the design and development of scaffolds for wound healing and tissue regeneration due to their remarkable qualities, such as biocompatibility, biodegradability and low toxicity [1]. The study of the possible interactions between these biopolymers has aroused and continues to have some interest in the biomedical field; the interaction between PL-PR could lead to the development of PL-PR composites as innovative biomaterials, easy to process, capable of assuming different geometries and sizes depending on the production method used [2]. Moreover, their functional properties depend on thecharacteristics of the constituent materials and the type of interactions. The interaction between PL and PRmay lead to the formation of both covalent and non- covalent PL-PR complexes. Electrostatic and hydrophobic interactions, Van der Waals forces andhydrogen bonds are the main driving forces for the formation of non-covalent bonds and, thus, the generation of coacervates or physical complexes [3].

The present work aims to produce new sustainable and high-value biomaterials, consisting of complexes between alimentary proteins such as whey protein isolate (WPI, containing more than 90% proteins) and chitosan (CS) or trimethyl chitosan (TMC), to be used for the manufacturing of scaffolds for skin repair. The starting materials, of which the complexes are made, derive from food industry waste. WPI are obtained from whey that is considered the most important environmental pollutant of the dairy industry, while CS is obtained by partial de-acetylation of chitin, that represents a major part of the seafood waste

Different complexes were prepared between WPI(Milei GmbH) and CS (Sigma Aldrich) or TMC (ChitoLytic), considering the following variables: PL molecular weight (MW), pH and WPI denaturation conditions, such as temperature and time.

The obtained PL-PR complexes were subjected to: (i) rheological measurements to point out the presence of a positive or negative rheological synergism ($\Delta\eta/\eta$), indicating the strength of the PL-PR interaction; (ii) turbidimetric analysis at 480 nm to investigate the formation of soluble complexes or coacervates (insoluble complexes); (iii) DLS and ELS measurements to determine complexes size and ζ potential; (iv) SEM analyses on freeze-dried PL-PR complexes to observe their micro/nanostructure. *In vitro* studies were also performed to investigate cell biocompatibility and proliferation enhacement properties of the complexes obtained, as well as their antioxidant activity. Moreover, the changes in WPI secondary and tertiary structures during denaturation and interaction with PL were also evaluated byintrinsic tryptophan fluorescence spectroscopy and free sulphydryl group content.

The in-depth characterization of the biomaterials highlighted that the functional properties of PL-PR complexes strictly depend on the biopolymer properties (MW, charge density and chain conformation) as well as on the experimental set-up (pH and denaturation conditions). More in details, it has been demonstrated that WPI denaturation at 70°C for 20 minutes, both before and after mixing with PL, was pivotal for the formation of PL-PR complexes. WPI denaturation before PL addition is responsible for the formation of soluble complexes, characterized by higher $\Delta\eta/\eta$ and lower absorbance values with respect to the values observed for coacervates, obtained as a result of WPI denaturation after PL addition. Moreover, rheological analysis highlighted that low MW CS was able to form stronger interactions with WPI than medium MW CS, under the same denaturation conditions. ELS analysis suggested that the electrostatic interactions are the main driving forces for TMC:WPI complex formation, while the intrinsic tryptophan fluorescence spectroscopy highlighted the prevalence ofhydrophobic interactions involved in the formation of CS:WPI complexes.

The innovative biomaterials obtained were used to prepare freeze-dried matrices with a sponge-like microstructure. They were characterized for mechanical, hydration and viscoelastic properties. These preliminary studies are pivotal for the use of the biomaterials in the design and development of platforms such as dissolving microneedles and films intended for hypertrophic scar remodeling and keloid treatment.

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POSTER N. 37

ENGINEERING OF DRUG DELIVERY SYSTEMS TO ENHANCE THE POTENTIAL OF DRUGS: FROM BEADS TO POLYMER-FREE NANOPARTICLES

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In this communication, the thirty-year research activity at the "*Drug delivery and targeting laboratory*" of the University of Modena and Reggio Emilia is briefly reported, highlighting the most innovative results. The research group, first directed by Professors R. Cameroni (co-founder of ADRITELF) and F. Forni, then by Valentina Iannuccelli and Eliana Leo with the collaboration of prof. G. Coppi, has developed, over the years, drug release systems of different dimensions (beads, microparticles and nanoparticles) and nature (polysaccharides, proteins and lipids) aimed to improve in vivo drug behaviour.

The production in the 90s of polysaccharide beads coated by using cross-linker inner residual is worth mentioning (1). Also, calcium alginate microspheres coated with chitosan to immobilize proteins (2) were estimated innovative and the published work is still cited as a pioneer in the use of chitosan in drug delivery systems.

In the mid-1990s, a delivery system based on gelatin nanoparticles for the delivery of doxorubicin was developed. The study demonstrated that doxorubicin remained partially bound to the protein matrix by the crosslinker (glutaraldehyde) and that the doxorubicin loaded nanoparticles were able to reduce the cardiotoxicity of the drug (3).

Between 2005 and 2010, solid lipid nanoparticles (SLN) as non-viral vectors for a model plasmid encoding the EGFP protein were also developed incollaboration with "Cell-lab" and the "Enzo Ferrari" Engineering Department of UniMoRE. The studies were among the first to demonstrate that cationic SLN are able to interact with large gene material such as plasmids. SLN cationized through the addition of protamine were able to allow the target of gene material up to the nucleoli (4).

In the context of collaboration on the project AIRC 2015 IG 16977 concerning the study of new compounds against ovarian cancer, liposomes and SLN have been developed for the delivery of an octapeptide against thymidylate synthase. The study showed that pH-sensitive liposomes can enhance the action of the peptide on immortalized ovarian cancer cells, allowing the cytoplasmic release of the drug (5).

A very intriguing line of research concerned the development of inhalable lipid nanoparticles for the pulmonary macrophage targeting of anti-tuberculosis drugs, such as rifampicin and isoniazid. The research was developed over several years and has benefited from national and international collaborations. Lipid nanoparticles decorated with a novel mannosylated surfactant capable of promoting active targeting to lung macrophages have been optimized (Italian patent n. 102017000123886). Alveolar targeting was demonstrated by *in vivo* translational studies of drug biodistribution in rats (6, 7). Also, in this field of breathable products, an innovative issue concerned the nasal administration of geraniol, a natural product known for its anti-inflammatory action and potential activity in the treatment of Parkinson's disease. The research was performed in collaboration with prof. Eliana M. Lima (Goiás, Brazil), and prof. A. Dalpiaz (Ferrara, Italy). The administration nose-to-brain of geraniol by SLN or cyclodextrins allows the brain retention of this compound in its active form (8, 9).

The most recent research aims to investigatetherapeutic activity of an endogenous compound endocannabinoid-like, Palmitoylethanolamide (PEA) that demonstrated anti-inflammatory activity but poor bioavailability. The potentiality of this compound in the treatment of sarcopenia by the production of PEA- loaded SLN and PLGA is currently under investigations (10). Finally, the formulation of PEA in the form of a polymer-free nanodrug has been patented and its development as cream against psoriasis was entrusted to PerFormS (www.performslab. it), spin off of UniMoRE.

Over the years the transition from basic to applied research characterized the activity of the "*Drug delivery and targeting laboratory*" team from Modena, thanks to the fruitful national and international collaborations that we hope will also be fundamental for future research.

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POSTER N. 38

DEVELOPMENT, CHARACTERIZATION AND PRECLINICAL ASSESSMENT OF A SPRAY DRIED N-ACETYL-L-CYSTEINE POWDER FOR INHALATION

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N-acetyl-L-cysteine (NAC) is a well-known mucolytic agent and it has antioxidant activity by acting as a scavenger of reactive oxygen species (ROS). It can be used to restore adequate levels of cysteine and glutathione (GSH) in the lung [1], thus enhancing host defence and cell protection mechanisms. The aging process leads to alterations in thiol levels and perturbs their redox state in the lungs, thereby increasing the risk of chronic diseases, such as asthma and COPD, and infections including SARS-CoV2. Over the past three decades, several studies highlighted the effects of NAC as an adjuvant in the treatment of viral infections, including its potential application in the treatment of SARS-CoV2 [2].

Since only NAC solutions are available for inhalation, the aim of this project was to develop, characterize and assess at preclinical level a NAC dry powder for inhalation. As far as we know, due to its sticky nature during the process [3], there were no successful attempts to produce a spray-dried powder of NAC alone.

All powders were produced by co-spray-drying using L-leucine (LEU) or mannitol (MAN) as excipients [4]. The effects of temperature, solvent and concentration were explored. NAC was quantified using a spectrophotometric method. The powders produced were characterized thanks to SEM, ATR-FTIR and DSC analyses. Aerodynamic performances were tested using a glass Twin Stage Impinger (TSI). The powder deposition on a transwell insert was also evaluated adapting the TSI configuration [5]. Tests were performed on both dry and wet insert. The same configuration was adopted to assess the best powder (NACi) effects on cell cultures. BEAS-2B cells were used as *in vitro* model of lung epithelium and they were exposed to 40 µM H_2O_2 to induce oxidative stress. Cell viability was measured by trypan blue dye exclusion and MTT test, and membrane damage was assessed by LDH leakage. Extracellular levels of H_2O_2 were measured as an indicator of cellular oxidativestress using the Amplex Red probe and catalase activity. IL-6 and IL-8 secretion was evaluated by ELISA and NFkB activation was studied byimmunoblot.

The best powders were obtained spraying water- ethanol (50:50 V/V) solutions using LEU as excipient. The best aerodynamic performances were obtained with NACi, a powder sprayed with 50% (w/w) LEU, at 40 °C, with 0.5% (w/V) solid content. The averageyield of three batches in the spray-drying process was 40%. NAC-MAN powders were characterized by low spray-drying yield and particle size incompatible with the inhalation route.

For NACi, the best NAC-LEU powder, SEM photomicrographs showed collapsed particles with a smooth surface.

FTIR spectra evidenced that NAC-MAN powders were characterized by the presence of MAN in a different polymorphic form compared to the raw material. In NAC-LEU powders, prepared from 0.5% (w/V) solutions, the FTIR analyses highlighted significant physical interactions but no chemical modifications were detected. For these powders, also DSC evidenced physical interactions. Indeed, there was a split of the NAC melting peak.

NACi was characterized by an emitted fraction of 68% and the respirable fractions of the emitted and of the nominal dose were 79% and 54% respectively. When adapting the transwell insert, differences were observed between the dry and wet insert. In particular, ~200 µg of NAC deposited onto the dry insert, while ~300 µg of NAC was found onto the wet one.

Cell viability data demonstrated that NACi has cytoprotective activity on H_2O_2 treated BEAS-2B cells. This effect was associated with a better control of H_2O_2 efflux from these epithelial cells and with an increased activation of catalase enzymatic activity. Furthermore, NACi treatment reduced IL-6 and IL-8 levels in the culture medium and NFKB activation in these cells.

In conclusion, spray-dried NAC powders for inhalation were successfully obtained and characterized.Preclinical data demonstrate that the newly developed NACi formulation holds great potential for inhalation treatments of lung inflammation and oxidative stress.

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POSTER N. 39

WHAT FUTURE LIES AHEAD FOR NANOMEDICINE?

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Since the first formulation of liposomes in the 1960s, nanomedicines (NMeds) have been at the forefront of innovative strategies for therapeutic and diagnostic application for overcoming the limitations of free drugs. Advances such as increased bioavailability, decreased off-target toxicity, improved biodistribution, protection of sensitive molecules, and penetration into hard-to-reach organs such as the brain [1-4], have led to several marketed NMed-based products for cancer therapy, e.g. Doxil® and Abraxane®, and the more recent Covid-19 vaccine Comirnaty® by Pfizer-Biontech [5]. Notwithstanding the increase in pharmaceutical potential and popularity as drug delivery systems, we are coming to an impasse as to the number of NMeds being considered for clinical trials due to difficulties in the design, characterization, scalability, and biological fate of the NMeds. This leads to the question: What is the future for NMed research?

CHARACTERIZATION AND REGULATORY ASPECTS

A crucial step before approval by regulatory agencies is a comprehensive characterization of the NMeds. It is now paramount to expand characterization beyond the standard methods (size, distribution, drug content, and stability) to better understand their influence on reproducibility and biological effect. With the use of novel materials and techniques, it is necessary to investigate the 3D-structure and composition of NMeds, including the amount of residual reagents and the internal/external organization. This can be especially difficult in the case of more complex systems, such as targeted NMeds: while the biological efficacy of surface engineered NMeds can be quantified by in vitro and in vivo tests [6], ligand quantification, localization, and conformation on the NMed surface is often overlooked. Nonetheless, these are critical aspects needed to better design NMeds that fall under regulatory approval guidelines.

MICROFLUIDIC PRODUCTION

One of the main limitations in the use of NMeds in clinics lies in the difficulties linked to their production at an industrial level. Scalability of traditional protocols, e.g. nanoprecipitation or thin layer rehydration etc., often represent a bottleneck that prevents the transition from laboratory production to clinical trials. Recently, novel techniques have been proposed to facilitate the translatability and scalability of production protocols, such as microfluidics (MF). Recent MF application to NMed research has become famous with its use in the global production of the Covid-19 vaccine. Thus, the current challenge is that of transferring already optimized protocols to a MF device [7], as differences in vitro have been demonstrated between NMeds created by standard and MF methodologies. Therefore, more in depth screening is needed to ensure consistency, reproducibility, and comparability for this new methodology.

BIOLOGICAL FATE

The biological fate of NMeds is one of the most complex, but most important aspects in determining therapeutic effect. Upon administration, a proteincorona is formed consisting of layers of plasmatic proteins adsorbed on the NMed surface that greatlyimpact their half-life, clearance, biodistribution, and toxicity [8]. Another recently discovered biological interaction is trafficking of NMeds via Tunneling Nanotubes which can bypass/circumvent the designed targeting strategies and lead to cell-to-cell transfer [9]. Thus, the future of applying NMeds will rely heavily on the research to better understand NMed interactions within the biological system such as blood components and cells, and cellular communication pathways.

DEPOT SYSTEMS

Target specificity and reduced off-target toxicity is one of the critical parts of NMed design, and NMeds alone are often insufficient to reach the goal. Therefore, hybrid systems are now being designed, where NMeds are embedded into an implantable system, as a complementary tool to meet all the requirements. Implantable systems, such as hydrogels [10] or sponges [11], show advantages in terms of long-term release and cell specificity, but their complexity often makes them difficult to fully characterize, posing novel challenges in this direction.

Research about NMeds is continuously growing: this poster will present some of the main aspects that need further investigation for reliable and marketable NMeds.

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POSTER N. 40

NANOMEDICINES FOR TRAUMATIC SPINAL CORD INJURY: DESIGN, OPTIMIZATION, AND *IN VIVO* EFFICACY

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Traumatic spinal cord injury (SCI) is characterized by a cascade of events that lead to sensory and motor disabilities. To date, this condition is irreversible, and no cure exists. Within 14 days after SCI, anti-inflammatory and pro myelinating drugs such as ibuprofen (Ibu) and T3 are critical to promote myelin repair and limit secondary degeneration, butoften are difficult to dose or insufficient for long term treatment (1,2). Nanomedicines (NMeds) can prolong the release profile of drugs, promoting tissue regeneration at the injured site (3).

Poly lactic-co-glycolide NMeds wereoptimized to load T3 and Ibu. A full chemico-physical, morphological, and technological characterization was performed, to assess size, polydispersity index (PDI), surface charge, morphology, amount of loaded drug, and release profile in artificial cerebro-spinal fluid.

Spherical NMeds containing T3 had a size around 160 nm and PDI ~ 0.1 indicating good homogeneity. The amount of T3 in the NMeds was calculated around 5% by weight, and had a prolonged release over 14 days (Figure 1A). Thus, the efficacy of T3-loaded NMeds was tested *in vivo* in SCI mice. Animals received a "combo" treatment consisting of a single injection of locally administered T3-loaded NMeds, and systemic Ibuprofen and Nerve Growth Factor for 14 days. Histological and behavioral tests revealed that the combinatory treatment was successful in restoring damaged tissues. Short term, a strong anti-inflammatory effect was noticed in the first few days. This was followed by an improve in remyelination that and reduction of the damaged area for weeks, with significant recovery in the behavioral tests of treated animals compared to control [4].

To further improve the therapeutic strategy and reduce the administrations needed, we investigated the encapsulation of Ibuprofen in the same NMed type. Ibuprofen had less positive results compared to T3: Ibu was immediately released from the NMeds (Figure 1B). Thus, to promote encapsulation, Ibu was conjugated to paracetamol (to form the conjugate IP), increasing hydrophobicity. NMeds of 150 nm, with PDI < 0.1 and almost 6% in weight of IP were formulated, achieving prolonged release of IP with eventual degradation into free Ibu and paracetamol (Figure 1C).

Optimized T3-NMeds improve the drug characteristics leading to improved motor impairment in SCI mice. To further improve this therapeutic approach, and to overcome the quick release of Ibu, IP was synthesized and successfully loaded into NMeds to reduce inflammation after traumatic SCI. Future work will focus on the complete release study of IP-NMeds and in vivo tests to assess the efficacy of both NMeds in SCI mice.

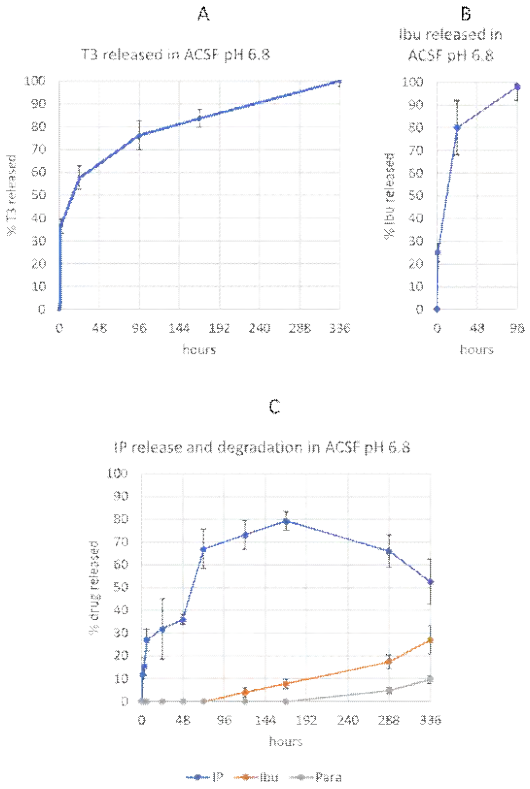

Figure 1: Drug release profile from NMeds loaded with A) T3, B) Ibu, and C) IP.

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POSTER N. 41

HYALURONAN-CHOLESTEROL NANOGELS FOR THE ENHANCEMENT OF THE OCULAR DELIVERY OF THERAPEUTICS

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The anatomy and physiology of the eye have always been a limit to the local delivery of therapeutics; cornea represents the main mechanical and chemical barrier limiting the diffusion of both hydrophilic and hydrophobic drugs into the anterior camera of the eye. Thus, the use of nanocarriers able to efficiently encapsulate therapeutics appears as an attractive strategy to facilitate the permeation and enhance ocular drug delivery [1-2].
Among all the polymeric nanocarriers that have been formulated to improve the bioavailability of ophthalmic drugs, polysaccharide-based nanogels (NHs) offer several advantages, such as biocompatibility, biodegradability and mucoadhesive property [3-4]. Since its abundance in the eyes, hyaluronic acid (HA) represents a good candidate for the preparation of NHs [5].

On this basis, an HA's amphiphilic derivative, obtained by grafting the polymeric backbone with cholesterol moieties (HA-CH), was used to load both hydrophobic (dexamethasone DEX and piroxicam PIR) and hydrophilic (tobramycin TOB and diclofenac DCF) drugs and ex vivo transcorneal permeation experiments were performed to assess the capability of such NHs to behave as permeation enhancers. It has been shown that the polymeric chains of HA-CH are able to spontaneously self-assemble in aqueous environment thus forming NHs by an easy-fast preparation procedure. Ex vivo studies by fluorescence microscopy and in-tube analyses with mucin showed that HA-CH NHs can interact with corneal components, being retained on porcine corneas, but they weren't able to penetrate the stroma.

Furthermore, DEX and PIR were successfully loaded into NHs with an encapsulation efficiency (%EE) of 57% and 37% respectively, thanks to the interactions with the internal hydrophobic cholesterol domains. The encapsulation of TOB was excellent (77% EE), due to the formation of electrostatic interactions between the positively charged drug and the negatively charged HA-CH NHs, whereas very low for DCF, which is negatively charged at physiological pH (14% EE).
Finally, ex vivo permeation experiments performed on porcine corneas showed that the permeation of the hydrophilic drugs is highly enhanced by NHs compared to the free drug solutions, whereas the permeation of the hydrophobic ones is strongly dependent on the water solubility of the entrapped molecules.

In conclusion, results suggest that NHs formulations can improve the ocular bioavailability of the instilled drugs by increasing their preocular retention time (hydrophobic drugs) or facilitating their permeation (hydrophilic drugs), thus opening the route to the application of HA-based NHs in the treatment of anterior eye segment diseases.

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POSTER N. 42

ENGINEERING TARGETED LIPOPLEXES FOR miRNA RESTORATION IN THE TREATMENT OF T-LARGE GRANULAR LYMPHOCYTE LEUKEMIA

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T-Large Granular Lymphocyte Leukemia (T-LGLL) is a rare hematological disease characterized by clonal expansion of cytotoxic T-Large Granular Lymphocytes (T-LGL) and associated with STAT3 activation by mutations of the STAT3 gene[1]. Neutropenia is a one of the major clinical hallmarks of this disease, which severely affect patient quality of life and life expectancy. Decreased levels of miR-146-b as consequence of STAT3 phosphorylation in T-LGL have been observed in patients, suggesting the potential benefit of the intracellular delivery of specific miRNAsas therapeutic approach for T-LGLL treatment[2].

We aim at developing biocompatible targeted lipoplexes for selective delivery and restoration of therapeutic miRNAs sequences to T-LGLL cells.

An innovative bioinspired oligocationic arginine rich enhancer (OCE) was synthesized according toprocedure developed by our group[3] and employed as condensing agent for the formulation of lipoplexes. Fusogenic lipids were included in the lipoplex composition to promote endosomal escape and cytosolic delivery of oligonucleotides. A library of lipoplexes was generated by varying the fusogenic lipid/total lipid and cholesterol/total lipid ratios withthe aim to identify the formulation with suitable biopharmaceutical and transfection performances. The library of lipoplexes was assembled by lipid film hydration with cy5 labelled oligonucleotides, using few different fusogenic lipids and N/P ratios in the 1-10 range, demonstrating that the OCE provides high loading efficiency (> 80% at N/P ratio 10) regardless ofthe lipid composition.

PEGylation of lipoplexes containing DOPE was performed by post insertion of $mPEG_{2kDa}$-DSPE on preformed lipoplexes in 0-7 $mPEG_{2kDa}$-DSPE/lipid mol% to provide for stealth features and explore a formulative space for the conjugation of anti T-Large Granular Lymphocytes antibody. The size and PDI of PEGylated lipoplexes were similar to their non-PEGylated counterparts, while zeta potential decreased with increasing $mPEG_{2kDa}$-DSPE/lipid ratio as consequence of the OCE shielding.

In-vitro cytotoxicity studies performed on a model cell line expressing GFP (H1299-eGFP cells) indicated that lipoplexes are devoid of cytotoxicity under the conditions used. Furthermore, cellular association studies demonstrated that the OCE facilitates the association of the lipoplexes with the cells. The therapeutic activity of lipoplexes loaded with oligonucleotides silencing eGFP expression was quantified by flow cytometric analysis. The results showed a good silencing of eGFP expression by the OCE-based lipoplexes, which was enhanced significantly with the inclusion of DOPE in the composition.

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POSTER N. 43

RATIONAL DESIGN AND DEVELOPMENT OF CONTROLLED RELEASE SYSTEMS FOR NEW GENERATION VACCINES

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Vaccines are a milestone among the prophylactic treatments against pathogen infections and are characterized by the highest cost-effectiveness benefits. Unfortunately, to achieve a sufficient immunization efficacy, multiple administrations are required, especially in infants and elderly people, which pose some limitations especially for some target populations (e.g pregnant women, infants) and in developing countries. A recent work demonstrates that the reduction of the number of administrations while preserving the vaccine efficacy can be obtained by mimicking the natural infection's exposure using a sustained antigen administration regimen. Reproducing a prolonged antigen exposure by using properly designed controlled release systems would provide for a better immune system activation and a higher immune response. Our study reported here focuses on focuses on the rational design and development of biodegradable and biocompatible polymers for the manufacturing of sustained antigen release systems for vaccines administration.

Preliminary *in vivo* experiments were set up in mice to identify the more suitable antigen exposure kinetic profile by studying different prolonged administration kinetics of the antigen ANT 1 (glycoconjugate targeting a bacterial disease) and ANT 2 (protein targeting a viral disease). In both the experiments the antigen dosage was fractionated and was administered with multiple injections or using osmotic pumps along one- or two-weeks exposure time. The results obtained from the assessment of IgG titres of recipient mice highlighted the higher or at least comparable production of antibodies titre with respect to classical prime and boost dose immunization regimen. In virtue of the promising results obtained by preliminary *in vivo* experiments, two different biodegradable Poly(lactcic-co-glicolic acid) (PLGA) microparticle (MP) manufacturing methods and the inclusion of selected matrix modifiers have been investigated to produce sustained antigen release systems. The first manufacturing approach was based on the classical double water/oil/water emulsion technique (CLL). The microparticles obtained by CLL were designed including excipients directly in the polymeric matrix to modulate both the antigen release and increase the antigen stability. For these purposes, Polyethylene glycol (PEG) and $Mg(OH)_2$ were included in PLGA matrix to accelerate the water intake in PLGA microparticle, favouring the PLGA hydrolysis, and to reduce the acidification related to the PLGA degradation which is detrimental for the antigen stability, respectively. A second innovative MP manufacturing method was aimed at producing porous antigen-free microparticles (nacMP) by double emulsion followed by antigen remote loading and the self-healing of the matrix. This approach has the aim to minimize the emulsion associated stress for the antigen. The MP were produced by including the matrix modifiers exploited in CLL formulations and positively charged lipids to increase the remote loading of the antigen. Microparticles with desired size were generated (i.e. 15 um). Both microparticle formulations obtained by CLL and remote loading/self-healing showed good and comparable antigen encapsulation efficiency according to the chromatographic and colorimetric assays. The antigens' stability after the release from microparticles was confirmed by HPLC-SEC or UPLC-RP chromatographic techniques and with ELISA assay. Finally, the PLGA microparticles were tested *in vivo* to investigate their ability of reproducing, by a single microparticle administration the immune response obtained by multiple dose administration regimen of vaccine.

Key words: vaccine; biodegradable microparticles; PLGA; sustained release; self-healing.

POSTER N. 44

SELENIUM NANOPARTICLES-DOPED PROLAMINES-BASED NANOFIBERS FOR WOUND HEALING VIA ELECTROSPINNING

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Prolamins are a group of proteins found in most cereal grains. They are biodegradable and sustainable sinceare obtained from natural sources. Moreover, they proved to be biocompatible and to have a great affinity with bioactive substances, and consequently promising to be applied in drug delivery and wound healing [1]. However, thay are scarcely soluble in water due totheir high content of nonpolar amino acids. Given this premises, the aim of this work is the design and the development of electrospun nanofibers based on zein or gliadin, blended with pullulan, a polysaccharide easily spinnable and characterized by excellent biological properties. The fibers were doped with selenium nanoparticles (SeNPs), an antimicrobial effective against *Pseudomonas aeruginosa*, one of the major pathogenic bacterium that could infect the skin wound [2-3].

Hydroalcoholic blends based on 15% w/w pullulan and 2% w/w zein or gliadin were prepared. Citric acid (2% w/w) was added to the polymeric blends and used as cross-linker. SeNPs were added to the polymeric blends to obtain doped fibers containing 0.5% w/w on dry weight.

The resulting blends were electrospun to obtain nanofibrous scaffolds, using a horizontal electrospinning apparatus (STKIT-40, Linari Engineering, Italy) equipped with a high voltage generator, a stainless-steel needle (0.8 mm), a volumetric pump (Razel R99-E) and a planar collector. The following parameters were used to obtain the scaffolds: voltage - 20 kV, needle-to-collector distance - 17 cm, flow - 0.8 mL/h, relative humidity 30-40%, temperature 25°C.

To improve mechanical properties and physical stability in aqueous environment, fundamental properties needed for scaffolds intended for skin implants, the scaffolds were crosslinked by heating 180°C/2h in an oven to obtain insoluble systems in aqueous environment.

Scaffold morphology was analyzed using scanning electron microscopy (SEM, Tescan, Mira3XMU, Czechia) after graphite sputtering, and nanofiber diameters were measured (Image J). The systems were characterized by nanometric dimensions and smooth surfaces. Moreover, the size of zein-based fibers was 3 times greater than gliadin-based fibers. Both the SEM analysis and the wettability, after crosslinking with citric acid, revealed the effectiveness of the crosslinking process in improving the hydrophobicity of the fibers. In particular, SEM analysis after hydration confirmed that the scaffolds retained theirnanofibrous structure, showing a slight swelling of the individual fibers.

The doping of SeNPs was investigated using transmission electron microscopy (Jeol JEM-1200 Japan). In both the scaffolds, the SeNPs were detected as black spots, having an electrondense structure higher than the polysaccharidic matrix that were dipped in. This supports that SeNPs were stable during the preparation of polymer blends and the electrospinning process.

The mechanical properties of the scaffolds werestudied using Texture Analyzer (Stable Microsystems, Italy). The fibers sheets obtained from the collectorwere cut into pieces of 1cm x 3cm (width x length) andsubjected to tensile force between the grips at a rate of 1 mm/s. The results showed that the mechanical properties after crosslinking increased significantly.

Currently, studies are ongoing to evaluate the effect of SeNPs on the chemical-physical properties of the fibers, moreover their biocompatibility and antioxidant properties *in vitro* on a model of human fibroblasts will be evaluated. Further investigations will be focused on the antimicrobial properties of the systems and to assess their efficacy in a murine excisional wound healing model.

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POSTER N. 45

MACHINE LEARNING AS A NEW APPROCH FOR THE DEVELOPMENT OF NOVEL ARYL HYDROCARBON RECEPTOR INDOLIC MODULATORS

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The Aryl Hydrocarbon Receptor (AhR) is a ligand-activated transcription factor that plays a crucial role in various cellular processes, including xenobiotic metabolism and immune regulation [1]. Indoles, a class of naturally occurring compounds, have been shown to interact with AhR and modulate its activity [2]. Recently, indole-3-carboxyaldehyde (IALD), our licensed AhR modulator with dual host-microbe activities, has been registered as orphan drug by the European Medical Agency. This has inspired further research on indolic compounds as a new avenue towards novel more potent AhR modulators to be further shaped up into suitable formulations for oral as well as inhaled therapies. However, proper approaches are needed to effectively explore target affinity andfunctional activity. In this work, a Machine Learning approach [3] was used to investigate indolic modulators. Machine learning (ML) is today in the spotlight for the performances in modeling complex systems as well as a powerful prediction capability. ML is a branch of artificial intelligence that focuses on the development of algorithms and models that enable computers to learn from data and make predictions or decisions without explicit programming. It involves theuse of statistical techniques to analyze and interpret patterns in large datasets, allowing machines to identifytrends, classify information, and make accurate predictions. By leveraging available experimental data on AhR activation and indole structures, severalmodels were trained and evaluated to accuratelyclassify indoles as AhR high and low activators. The dataset consisted of a matrix composed of a diverse collection of indole derivatives, their molecular descriptors and corresponding AhR activation data. Various ML algorithms, including Classification and Regression Trees (CART), k-Nearest Neighbors (kNN), Neural Network (Nnet), Support Vector Machines (SVM) with a linear kernel, and Random Forest (RF) were employed to model the complex relationship between the indole structures versus the corresponding AhR activation (Table 1). The developed models exhibited excellent predictive performance, achieving high accuracy (about 82%), sensitivity (92%), and specificity (70%) in classifying indoles as AhR high or low activators. Thus, the identified key structural features shed light on the molecular determinants crucial for AhR activation by indoles, aiding in the design of novel compounds with desired AhR-modulating properties. These findings highlight the potential of integrating ML approach in predicting AhR activation by indole derivatives, providing a valuable tool for rapid screening and prioritization of compounds for further experimental investigation.

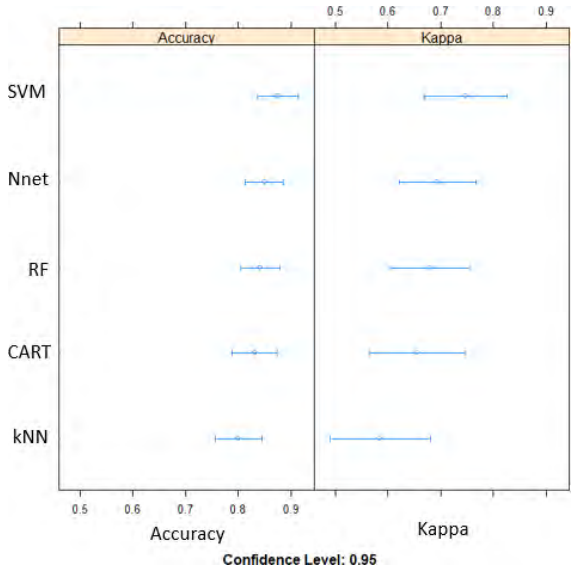

Figure 1. Accuracy and Kappa of different algorithms: SVM, Nnet, RF, CART, kNN.

This research step is aimed at providing a tool for further implementation of screening techniques for indolic AhR ligand investigation and identification. This will help to expedite subsequent development of inhalation and oral delivery platforms to be translated for product commercialization.

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POSTER N. 46

WETTABILITY AND HYDRODYNAMICS KEY HALLMARKS ON DRUGS' DISSOLUTION RATE

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DRT (Dissolution Rate Test) is an essential test, widely used in the pharmaceutical field [1]. It is meant to evaluate the *in vitro* dissolution kinetics of a mix of poly-dispersed drug particles in water or physiological fluid. This is a key drug hallmark, which is strictly connected to its main properties such as drug solubility, wettability, particles shape and size distribution. Therefore, DRT correlates with the *in vivo* drug dissolution behavior, i.e. the *in vitro-in vivo* links. Indeed, an important aspect in the development of pharmaceutical products involves its *in vitro* characteristics and related *in vivo* performance.

Herein, the present work focuses on the most important phenomena occurring within DRT experiments and related mathematical modelling. The dissolution phenomenon is associated to four consecutive steps [2], namely: 1) contact of the solvent with the solid surface (*wetting*), 2) breakdown of intermolecular bonds in the solid phase (*fusion*), 3) molecules transfer from thesolid phase to the solid/liquid interface (*solvation*), 4) diffusion of the solvated molecules through the unstirred boundary layer surrounding the solid surface (*diffusion*). These steps represent the total resistancefor the drug molecules to move from the solid phase to the solution one (dissolution). Moreover, the first three steps (the first one is ruling out) are connected to the surface resistance (R_m) for a drug dissolution, while thelast one (R_d) depends on the thickness (δ) and the drug diffusivity related to the unstirred boundary layer surrounding each particle. Thus, Fick's second law is required, to mathematically connect the four steps, assuming stationary conditions within the boundary layer. This allows to obtain the time variation of the drug profile concentration inside the unstirred layer. Particles are assumed to be spherical, with radius R, thus δ depends on particle dimension. We hypothesize that particles can be distributed into different size groups: each of them must comply with Fick's second law. By merging Fick's second law with the equation describing particle radius reduction for each particle class [3] and the overall mass balance on the dissolution environment, it is possible to gain the time variation of the drug concentration (C_b) inside the liquid phase. Two different model drugs have been studied: theophylline and praziquantel. The first one is easily wettable and it does not show solubility problems (class I of BCS [4]), whereas the second one is a typical BCS class II drug, showing also wettability issues, as per both the water contact angle and the solidvapour surface tension.

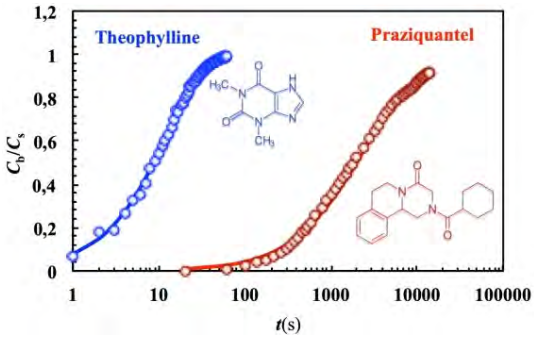

Figure 1. DRT of the two drug considered. t is time and C_s is drug solubility. Solid lines indicate the model best fitting.

Figure 1 reveals a perfect match between experimental data and model fitting. Moreover, model outcomes indicates that $R_{m\text{-PRAZIQUANTEL}}$ is about 10^2 times that of theophylline and this is the main reason why praziquantel dissolution is so slower compared to the theophylline one.

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POSTER N. 47

MICROFLUIDIC-ASSISTED PREPARATION OF SOLID LIPID NANOPARTICLES FOR THE BRAIN-DELIVERY OF BIOLOGICALS: AN *IN VITRO* EVALUATION

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Traumatic brain injury (TBI) is a comprehensive term that refers to a number of impairments triggered by trauma affecting the brain. Since TBI is identified as the leading cause of deathand long-term disability in children, it appears to be a severe public health concern worldwide [1]. The pathological manifestation of TBI involves first a physical injury, which results in an increased permeability of the blood-brain barrier (BBB), and thena significant release of pro-inflammatory mediators, which leads to a severe neuroinflammation event [2]. During this detrimental phenomenon, the breakdown of the BBB creates the opportunity for passively directing non-invasive drug delivery systems (DDSs) to the brain in order to administer neuro-protective and/or neuro- regenerative agents [3]. Current scientific research indicates that neurotrophins are important for the repair of injured brain parenchyma, and more specifically, the brain-derived neurotrophic factor (BDNF) could lead to beneficial regenerative effects [4]. However, BDNF is a pH-sensitive molecule with a short half-life, so it would necessitate exceedingly intrusive methods for its administration when following a conventional treatment plan [5]. Therefore, the application of novel DDSs for the brain delivery of BDNF would circumvent pharmacokinetic restrictions, allowing for the treatment of secondary neurodegenerative phenomena through more compliant processes [6]. In this field, the solid lipid nanoparticles (SLNs) represent a promising DDS for biologicals; in fact, they showed valuable properties as the ability to protect the sensitivecargo from the rapid *in vivo* degradation. Furthermore, by exploiting novel strategies as the microfluidic technique (MF), it has been demonstrated the feasibility of high-quality nanosystems while keeping the biological activity unaltered [7].

Here, BDNF-SLNs have been successfully obtained by MF which ensured the achievement of monodisperse nanosized DDSs. The production has been conducted through the before-optimized method [7] using a commercially available device with a miniaturized modified herringbone geometry. Characterization data about the freshly produced BDNF-SLNs showed the proficiency of MF-based production; in fact, this method allowed to achieve good findings in terms of dimensional range (190.3 ± 10.1 nm), PDI (0.180 ± 0.023), and ζ-potential (– 39.2 ± 1.30 mV), resulting in an encapsulation efficiency (EE %) equal to 40.3 ± 2.7 %. The evidence that BDNF-SLNs exposed a homogeneous negative surface charge was indicative that the growth factor was protected within the lipid core and not absorbed on the nanosystems' surface [7]. Then, in order to compare the permeability of encapsulated BDNF and plain one, an *in vitro* BBB model has been built with a monolayer of immortalized human endothelial cell line (hCMEC/D3). Interestingly, after 3 hours of permeation across the cell monolayer, collected data demonstrated a slightincrease in the P_{app} of the encapsulated BDNF (1.27×10^{-5} cm/sec) in comparison with plain BDNF(9.31×10^{-6} cm/sec) at the same concentration of growthfactor, namely 3.98×10^{-3} μg/mL. For evaluating the potential therapeutic benefits of BDNF-SLNs in a TBI-like condition, an *in vitro* microglial cell model was built using the N9 cell line. Treatments with BDNF- SLNs and plain BDNF at concentrations, 25 ng/mL and100 ng/mL, in terms of BDNF, were performed for 4 hours before the addition of lipopolysaccharide (LPS, 1 μg/mL) and LPS + interferon γ (IFN-γ, 0,1 μg/mL) and

1 hour after the addition of LPS and LPS+INF-γ. Empty SLNs were used as control. At the end of experiment, the amount of nitrites as NO products was measured to evaluate the neuro-protective effect of BDNF. In addition, real-time PCR analysis was conducted to follow the production of pro- inflammatory agents. As a result, data showed that the formulation of BDNF-SLNs (100 ng/mL) was capable to reduce the iNOS activation resulting in less nitrite production in each tested condition compared to plain BDNF at the same concentration. Regarding real-time PCR results, the samples in which the toxic insult was added 4 hours after treatment with BDNF-SLNs (100 ng/mL) showed a complete elimination of pro- inflammatory TNF-α cDNA compared to empty SLNs and plain BDNF. Eventually, BDNF-SLNs (100 ng/mL) highlighted potential neuro-protective activity in a TBI-compromised condition, opening the way for further evaluation.

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POSTER N. 48

DIETHYLDITHIOCARBAMATE-COPPER COMPLEX NANOPARTICLES FOR BREAST CANCER TREATMENT

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INTRODUCTION

Disulfiram, the anti-alcoholism drug approved by FDA in 1951, has also shown a remarkable anticancer activity[1]. Within its metabolites, diethyldithiocarbamate-copper complex ($(DDC)_2$-Cu) has been recognized as the main responsible for disulfiram antitumor activity[2], which derives from a combination of multiple molecular mechanisms that are effective against differentiated cancer cells and also against cancer stem cells[3].

Although disulfiram seems to be highly cytotoxic to cancer cells *in vitro*, the short half-life in the bloodstream[4], the low selectivity for cancer cells over healthy cells and the water insolubility limits its efficacy *in vivo*. Thus, $(DDC)_2$-Cu encapsulation in drug delivery systems may represent an efficient strategy to overcome these limitations[3].

Herein, a lipid and polymer-based drug delivery systems for the administration of $(DDC)_2$-Cu were developed and compared.

MATERIALS AND METHODS

Polymeric nanoparticles (NPs) were formulated by nanoprecipitation of a drug and polymer solution in dimethyl sulfoxide into water using a 1:1 mixture of poly (D, L-lactide co-glycolic) acid (PLGA, Resomer® 503H, M_w 24-38 kDa) and PLGA-PEG_{2kDa}. Solid lipid nanoparticles (SLNs) were formulated throughmicrofluidic device (NanoAssemblr Benchtop), by mixing,in a 3:1 ratio, DI water to an organic phase containing cholesterol, Hydrogenated Phosphatidyl Choline fromSoybean (HSPC) and $(DDC)_2$-Cu.

Drug loaded-nanocarriers size and zeta-potential were analyzed by Malvern Zetasizer Ultra. Nanocarriers morphology was evaluated by Trasmission Electron Microscope (TEM).

Stability studies were performed by incubating 1 mg/mL polymer or lipid nanocarriers at 4°C in DI water,Phosphate Buffer Saline (PBS) or 5% Fetal Bovine Serum (FBS) in PBS. Release studies were performed at 37°C at a 3.5 mg/mL concentration for SLNs and 2 mg/mL of NPs.

Free $(DDC)_2$-Cu and $(DDC)_2$-Cu loaded PLGA-PEG_{2kDa} NPs and SLNs were tested for their anticancer activity against MDA-MB-231 and MCF-7 breast cancer cell lines by MTS assay.

RESULTS AND DISCUSSION

PLGA-PEG_{2kDa} NPs and SLNs were formulated choosing a hydrophobic material, either PLGA or cholesterol, toform a core suitable for $(DDC)_2$-Cu encapsulation, and an outer layer that could provide stealthing or interfacial properties to the system: PEG_{2kDa} and HSPC. DLSanalyses revealed that both systems have a homogeneous size of 172.1 ± 8.1 nm and 106.9 ± 5.79 nm with a PDI of 0.035 ± 0.020 and 0.103 ± 0.030 for PLGA-PEG_{2kDa} NPs and SLNs, respectively, and an almost neutral zeta-potential. TEM analyses, on the other hand, showed a spherical shape and a size of 92.7 ± 1.72 nm for PLGA-PEG_{2kDa} NPs and 82.62 ± 0.03 nm for SLNs. PLGA-PEG_{2kDa} NPs showed a much higher encapsulation efficiency percentage (EE%) as compared to SLNs (60.9 ± 16.5% and 7.22 ± 1.22%, respectively).

Stability studies revealed that PLGA-PEG_{2kDa} NPs were stable at 4°C at least for 4 weeks in presence of PBS, and they did not interact with serum proteins at least for 48 hours in 5% FBS in PBS. Release studies showed that 23%of $(DDC)_2$-Cu was released from NPs within the first 8 hours, and more than 50% of the encapsulated drug in the 10 days of monitoring. SLNs, on the contrary, were unstable in PBS showing aggregation at 4 h, yet serum proteins could interact with their surface, preventing this phenomenon. Instead, SLNs were stable in water at 4°C in the two weeks of monitoring. When incubated at 37°C in aqueous environment, SLNs did not release $(DDC)_2$-Cu in 48 h, suggesting that the drug is strongly retained in the solid bulk of the nanoparticles.

Finally, free $(DDC)_2$-Cu and $(DDC)_2$-Cu loaded PLGA-PEG_{2kDa} NPs and SLNs were tested for their anticancer activity against MDA-MB-231 and MCF-7 cell lines. In all the tests performed, both free $(DDC)_2$-Cu and loaded nanocarriers showed a concentration-dependent cytotoxicity. Furthermore, the cytotoxic activity was displayed at low concentrations of $(DDC)_2$-Cu. In MDA-MB-231 cell line, free $(DDC)_2$-Cu was as active as loaded PLGA-PEG_{2kDa} NPs, while SLNs were slightly less active (IC_{50} values of 0.23 and 0.28 μM, respectively). Instead, in MCF-7 cell line PLGA-PEG NPs and SLNs were more active than free $(DDC)_2$-Cu (IC_{50} values of 0.21 and 0.23 μM for nanocarriers and free drug, respectively). Thus, $(DDC)_2$-Cu confirmed its cytotoxic activity against breast cancer cell lines, including phenotypes that are highly aggressive and with low differentiation, such as MDA-MB-231. Hence, the encapsulation of $(DDC)_2$-Cu in drug delivery systems is a promising strategy for improving its delivery.

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POSTER N. 49

CO-DELIVERY OF ICARIIN AND NAPROXEN BY OLEOSOMES FOR POTENTIAL TOPICAL ANTI-INFLAMMATORY APPLICATION

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Inflammation is the underlying cause of several physiological and pathological processes. In recent years, biocompatible topical nanocarriers for inflammation have piqued the scientific community's interest [1]. Icariin (ICA), considered to be the active compound in the herb Epimedium (also known as Horny Goat Weed), is a notably effective natural drug that mediates anti-inflammatory effects such as downregulation of TNF- α, PGE(2), and Nitric oxide (NO), as well as suppression of NF-κB p65 activation[2]. Nonsteroid anti-inflammatory drugs (NSAIDs) are the class of drugs that are used most frequently due to their effectiveness in relieving pain and inflammation [3]. Naproxen sodium (Nx) is anover-the-counter (OTC) nonsteroidal anti-inflammatorydrug (NSAID) that is commercially available as a sodium derivative. Unfortunately, as well as Icariin, it lacks the physicochemical properties that are ideal for cutaneous and transdermal permeation, thus limitingthe topical anti-inflammatory effect [1].

The aim of this study was to realize an ultradeformable nanosystem able to deliver both ICA and Nx in order to improve the percutaneous permeation and obtain a synergistic anti-inflammatory effect. We investigated different lipid-based nanovesicle systems such as Liposomes, Transferosomes, and Oleosomes. Oleosomes was considered a versatile system useful for topical application among these nanovesicles. Olesomes has been reported to enhance dermal permeability, making them a viable topical therapy [3]. Different formulations of oleosome with a molar ratio of phospholipids (PL90G) and oleic acid from 1:1 to 2:1 (mol ratio) and different concentrations of both drugs from 1 mg/ml to 4 mg/ml were investigated in order to get stable nanovesicles. The best results were obtained using 1 mg/ml of both drugs, showing an effective retention of approximately 50% for both ICA and Nx. In this investigation, the optimized oleosomes had suitable physiochemical properties with particle size(151 nm), polydispersity index (PDI) 0.07, Zetapotential (-56 mV) and physical long-term stability,thus aiming at assessing its potential efficacy as a topical treatment for inflammatory affections. Additionally, kinetic release profiles showed asustained release of both drugs for up to 72 hours. In particular, biphasic profiles were recorded and a steady state was reached after 24 and 48 hours for ICA and Nx, respectively.

Resulting drugs-loaded oleosomes were tested in vitro on NCTC cells, demonstrating the absence of cytotoxic effect up to the drug concentration of 20 µM. Furthermore, the deformability test demonstrated suitable physicochemical features of nanovesicles after the passage through a pore size of 50 nm. The use of oleic-based nanosystems also revealed an improved permeation of both drugs in an ex-vivo study by using a human stratum corneum in a Franz-cell study.

Based on the mentioned results, the potential application of the proposed nanovesicles as advanced carriers for the delivery of both natural and synthetic drugs, may increase the therapeutic efficacy of payloads for the topical treatment of inflammatory diseases.

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POSTER N. 50

FROM MUCOADHESION TO TISSUE ENGINEERING: A JOURNEY DRIVEN BY THE RESEARCH ON MULTIFUNCTIONAL AND BIOACTIVE POLYMERS

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Introduction

The history of the research group starts in the 90s under the direction of Prof. Carla Caramella with the study of the mechanisms of the mucoadhesion phenomenon [1, 2]. In those years the group published many papers focused on the investigation of the interaction between mucins, the main component ofmucus, and some natural polymers such as cellulose derivatives, chitosan (CH) and its derivatives, and hyaluronic acid (HA). Some of them, namedmultifunctional polymers, proved also to interact with the mucosal epithelium by disturbing intercellular junctions and acting as penetration enhancers or by promoting cell proliferation and enhancing tissue repair[3]. In 2000s the national grants received and the collaboration with researchers of IRCCS San Matteo Hospital (Pavia) have directed the research of the group on the use of such multifunctional and bioactive polymers for the development of therapeutic platforms intended for tissue repair, focusing in particular on the treatment of mucosal lesions, skin ulcers, tendons damages and nerve injuries.

In the present abstract the main results of the research of the last five years on such topics are summarized.

Mucosal lesions

The first papers of the group on tissue repair were focused on the development of mucoadhesive formulations for the treatment of buccal and vaginal mucositis. A patent on the combination of bioadhesive polymers with platelet lysate (PL), a hemoderivate rich in growth factors, was also granted [4].

In the last years, mucoadhesive and ion- and/or thermo-sensitive in situ gelling systems have been developed. They were based on a proper combination of κ-carrageenan or methyl cellulose, as multifunctional polymers able to undergo sol-gel transition upon administration**,** cellulose derivatives as mucoadhesive agents and xyloglucan as moisturizing excipient.

The occurrence of a synergistic effect among polymers in terms of rheological properties and capability to interact with mucosa was functional to prolong the permanence time of the formulations at the application site and to guarantee a protective action towards ulcerative lesions from additional damages [5, 6]. Moreover solid systems, nanofibrous scaffolds based on polysaccharides (CH, alginate (ALG) and gums)and proteins (gelatin (GL) and fibroin) have been desiged to be implanted into the periodontal pocket to restore tissue integrity [7].

Skin ulcers

The therapeutic approaches for the management of difficult-to-treat wounds have undergone over the years a progressive change: inert dressings have been replaced by bioactive ones, based on biopolymers able to interact with tissue components and take part in the healing process. The research of the group has followed this evolution, developing different formulations (films, sponge-like and particulate systems, nanofibers) based on biopolymers able to restore native functions of the damaged skin [8].

Dressings able to guarantee a combined delivery of PL and an anti-infective model drug, vancomycin hydrochloride (VCM), in the chronic ulcers were developed [9]. A simple method was set up for the preparation of HA core-shell particles, loaded with PL and coated with calcium ALG, embedded in a VCM containing ALG matrix.

Subsequently innovative approaches aiming at the development of green and sustainable process were developed and physical crosslinking achieved to avoid chemicals. At this purpose electrospinning was used to prepare nanofibrous membranes entirely based on polysaccharides, starting from aqueous polymer blends [10]. CH/chondroitin sulfate scaffold loaded also with Ag nanoparticles, nanocomposites and clays, proved effective in preclinical models in vivo (murine burn/excisional model) and in vitro (fibroblasts and HUVEC) [11-14].

Electrospun nanofibrous scaffolds based on maltodextrin and α-amino acids cross-linked via Maillard-type reaction were designed [15]. This crosslinking conferred to the scaffolds distinctive properties as antioxidant ones due to the presence of melanoidins that possess a strong antioxidant activity.

Smart nano-in-microparticles based on polysaccharides (maltodextrin or dextran) and amino acids, and doped with antibacterial nanoparticles (CuO or ZnO NPs) were developed [16]. The resulting 3D structures proved to control tissue moisture and to act as a scaffold to enhance cell migration from the surrounding healthy tissue, cell adhesion and proliferation leading to the reconstruction of the architecture of native skin.

Tendon damages

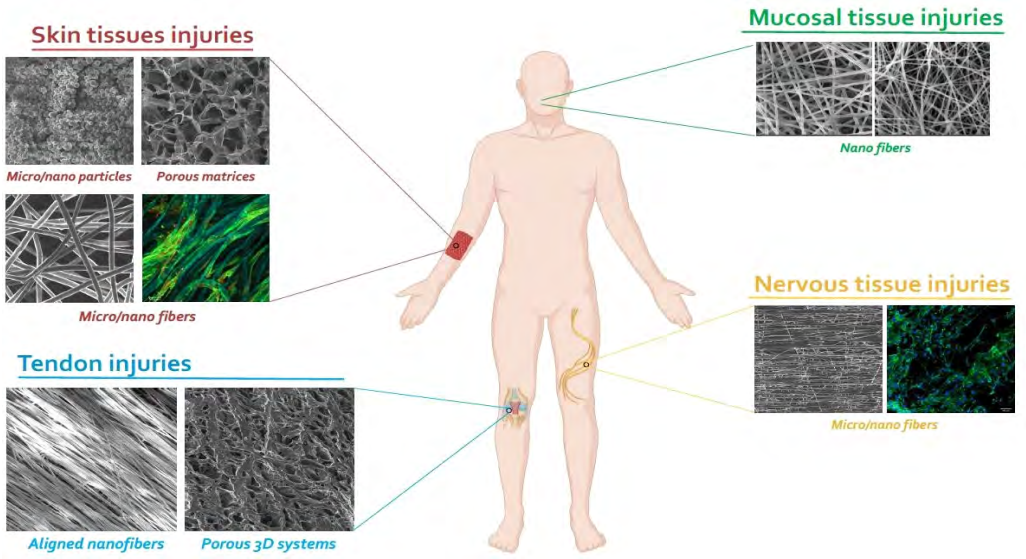
More recently the research group explored also the orthopaedic field and polymer-based scaffolds having the ability to mimic the structural, biomechanical and biochemical functions of the extracellular matrix and the native tendons were designed. Electrospinning and freeze-drying were used to manufacture porous and aligned nanofibers and 3D scaffolds. Natural and synthetic polymers were doped with inorganics to increase the mechanical properties and the

biocompatibility, improving cell adhesion, proliferation and differentiation and tissue healing potential [17].
Electrospun hybrid tubular scaffolds were enriched with hydroxyapatite nanoparticles and extemporaneously loaded into the inner cavity with PL, with the aim of leading to complete post-surgery functional regeneration of the tissue. The scaffold obtained showed tendon-like mechanical performance, enhancing tenocytes and osteoblasts adhesion and proliferation [18].
Nanofibrous scaffolds based on poly (butyl cyanoacrylate), a well-known biodegradable and biocompatible synthetic polymer, doped with copper oxide nanoparticles and caseinphosphopeptides, were developed as implants to be sutured to reconstruct the tendons and the ligaments during surgery [19]. The obtained scaffolds proved to be biocompatible towards tenocytes and characterized by anti-inflammatory and antibacterial properties.

Nervous tissue injuries

In the last five years, the research group has spentefforts in the design and development of formulationintended for the treatment of spinal cord (SCI) and peripheral nerve (PNI) injuries.

Nanofibers and freeze-dried polymeric matrices based on CH, ALG and gellan gum (GG) have beendeveloped for nerve tissue regeneration [20, 21]. Inparticular, in recent works, the use of spermidine (SP), an endogenous polyamine, was investigated in association with ALG or GG for the development of nanogels and nanofibers. SP showed a dual role, acting as cross-linking agent for ALG and GG and as antioxidant and anti-inflammatory compound having a neuroprotective effect. GG-SP based nanofiberscontaining GL were developed. Thanks to GG-SP interaction, fibers were insoluble in aqueous medium and characterized by high mechanical properties. Nanofiber biocompatibility was evaluated on Schwann cells, showing that the presence of GL enhanced nanofibers compatibility with cells [22].
Micro/nanogels based on the ionic interaction between ALG and SP were obtained via ionotropic gelation. Different ALG concentrations and viscosity grades and different SP concentrations were considered. In vitro studies on Schwann cells proved the ability of SP of expressing antioxidant and anti-inflammatory properties, even if involved in the formation ofnanogels [23].



Schematic representation of the research topics

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POSTER N. 51

3D-PRINTED PATCHES BASED ON BROMELAIN AND ALOE VERA FOR WOUND HEALING APPLICATIONS

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The present research study was focused on the development of a 3D-printed patch for wound healing applications and based on the bioactive compounds present in Bromelain and Aloe Vera.
In literature, several studies report on the efficacy of these extracts in the wound healing management.
Bromelain can be beneficial for debridement of necrotic tissue and acceleration of healing thanks to thepresence of escharase, which is responsible for this effect. Escharase is nonproteolytic and has no hydrolytic enzyme activity against normal protein substrate and in different enzymatic debridement studies showed rapid removal of the necrotic layer of the dermis with preservation of the unburned tissues [1].
Aloe vera (Aloe barbadensis) preparations have been used for centuries to treat wounds and burns. Aloe vera gel is an extremely complicated mixture of natural products, but the biological activity is principally attributed to polysaccharides and glycoproteins present in the leaf pulp. Acemannan, the main polysaccharide present in aloe vera gel, plays an important role in the wound healing process by inhibiting bacterial growth and stimulating macrophage activity [2].
For this reason, Bromelain and Aloe Vera were used as an alternative to synthetic drugs to develop active wound dressing patches useful for wound healing applications.
The patches were developed using two different pre-crosslinked gels composed by Chitosan and Alginate, as natural polymeric materials, and Tripolyphosphate and Calcium Chloride, as crosslinking agents. In addition, Glycerol was added as plasticizer in the aim to improve the mechanical performances.
For a deeper structural understanding of pre-crosslinked gels and relating their properties with the Semi Solid Extrusion (SSE) 3D-printing process, rheological analyses were carried out demonstrating stronger mechanical integrity and shape retention for both gels. These properties are desired during an SSE process and may help in the preparation of 3D-printed skin delivery patches [3].
The 3D model was designed through Rhinoceros 6 CAD software and SSE 3D printing process was developed using a home-made 3D printer. It stood out by providing a simple and rapid method for the production of batches with adjusted shapes and sizes [4].
The obtained patches were, therefore, characterised in terms of antioxidant and anti-inflammatory activity and the conducted colorimetric tests showed a notable scavenger action of the samples on DPPH, $ABTS^{\cdot+}$ and $NO\cdot$ radicals, significant total phenolic content and total antioxidant capacity.
In vitro release studies were carried out at different pH (5.5, 7.4 and 9.0) in order to verify the release of Bromelain under the different conditions that characterise burn wounds [5]. Furthermore, the Bromelain enzymatic activity was evaluated and it was observed that it increased with a constant and similar trend to that observed in the release profiles. This result shows how the incorporation of the active ingredient into the polymeric patches does not degrade the enzyme, which is gradually released, maintaining its activity over time and at different pH levels.
Subsequently, the patches were characterised with regard to exudate absorption capacity, degree of degradation, water vapour transmission rate and porosity, showing behaviour suitable for use in wound healing applications.
Finally, the results of the cell viability studies demonstrated high cell viability rates in the presence of the prepared patches, indicating their ability to support cell growth and survival. Additionally, the *in vitro* scratch test revealed that the patches not only exhibited biocompatibility but also displayed remarkable effectiveness in promoting the healing of scratches.

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POSTER N. 52

OPTIMIZATION OF AMBISOME® THERAPY MANAGEMENT IN A HOSPITAL PHARMACY

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Amphotericin B (AmB) is an antibiotic that belongs to the class of polyene macrolides, isolated in 1955 from *Streptomyces nodosus*. It is used for the treatment of various types of deep and systemic mycoses but is not effective against bacterial microorganisms [1]. AmB is a yellow/orange product slightly soluble in water, with a tendency to self- associate in tight aggregates due to its amphiphilic nature. Once intravenously administered, AmB exerts its therapeutic effect along with adverse reactions causing anemia, nephrotoxicity, and anaphylaxis. The pharmacokinetics, toxicity and activity strictly dependent on the type of AmB formulation. Encapsulation in drug delivery systems, such as liposomes, nanoparticles and microspheres resulted in high concentrations of AmB in liver and spleen, but lower concentrations in kidney and lungs, so decreasing its toxicity. Among these formulations, those recommended for parenteral use by AIFA areliposomal AmB (AmBisome® 50 mg powder for concentrate and solution to infusion), AmB deoxycholate (Fungizone® 50 mg powder for intravenous solution), and AmB phospholipid complex (Abelcet® 5 mg/mL concentrate for intravenous suspension) [2].

Up to date, AmB still remains the gold standard for the treatment of neonatal candidiasis and for this reason its use in a pediatric hospital, G. Gaslini Children Hospital (Genoa, Italy), has drawn much attention, concerning in particular the management of the different formulations for hospital use.

From May 2022, Gaslini's hospital pharmacy has considered to restrict the preparation of AmBisome to pharmacy (i.e., unavailable vials in floor stock) with the aim of better preserving a checked system, and to optimize dispensing and scheduling.

This research aims to study the centralized methods of AmBisome® reconstitution [3] and to evaluate its stability under in use-conditions, in terms of active principle amount and changes in nanoparticle characteristics linked to the intrinsic properties of liposomes.

In particular, we explored the colloidal stability and the cytotoxic activity of AmBisome® formulations, both in sterile water or in 5% glucose solution maintained at room temperature or at 4 °C, up to one week from reconstitution. In order to establish if the procedure undertaken led to a minimization of the costs, the number of vials dispensed for two months before and after the decision of using the pharmacist-provided medication therapy management approach, were analysed.

The lyophilized powder was reconstituted with 12 mL sterile water so as to obtain a drug concentration of 4 mg/mL following the SmPC instructions with slight modifications (according to the hospital pharmacy practices). Then, the liposomal suspension was vortexed for 30 s until aggregates dissolved and homogeneity was reached. The infusion to be administered in patients was obtained by transferring an aliquot of the suspension with a sterile syringe equipped with a 5 µm filter into an infusion bag containing 5% glucose. Volume was previously adjusted to reach a final drug concentration rangingfrom 0.2 to 2 mg/mL, according to patient's weight.

The aggregation states of AmB were assessed by UV–visible spectrophotometry, measuring the absorbance at two characteristic wavelengths: 325 nm and 408 nm, attributable to the oligomeric state and to the monomeric state, respectively. Quantitative assays were performed using a modified RP-HPLC method.

Mean diameter (Z-average), polydispersity index (PDI), and Zeta potential (ζ) of the liposomal formulations were recorded using a Malvern Nano ZS90 light scattering apparatus and the *in vitro*colloidal stability of reconstituted formulations was also studied by Nanoparticle Tracking Analysis using aNanoSight NS 300.

Preliminary results evidenced that the formulations kept in water at 4°C showed an increase over time in liposomes mean diameter (from 87.0±1.0 nm to 104.0±3.5 nm) suggesting a possible drug leakage. PDI and ζ remained almost constant, 0.210±0.007 and -32.4±7.2 mV, respectively. The analysis of the AmB aggregation state revealed modifications in all the formulations kept at room temperature, while HPLC analysis highlighted the onset of two peaks after 24 h storage at 4°C.

These results could suggest some adjustments in pharmacy practices regarding Ambisome® management, in terms of drug storage and in use-conditions and also in regards of the centralized individual doses preparations by the hospital pharmacy.

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POSTER N. 53

CYCLODEXTRIN-BASED SUPRAMOLECULAR DEEP EUTECTIC SOLVENT (CYCLODES): A NOVEL SYNERGIC APPROACH FOR POORLY SOLUBLE DRUG DELIVERY

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Deep eutectic solvents (DES) have gained more and more interest in the past years as a novel and green approach in chemistry, mainly for their versatility and for being synthesized from readily available materials characterized by hydrogen bond acceptor and donor moieties. These mixtures are prepared by heating and mixing two or more compounds, usually solids at room temperature (RT), at a specific molar ratio, resulting in a clear crystal liquid even at low temperatures [1].

The advantages of DES can be easily resumed in nonreactivity with water, low- or nontoxicity, biodegradability, ease of preparation, and inexpensive and readily available constituents, often belonging to the generally recognized as safe (GRAS) list [2].

Herein, taking advantage of hydroxy-propyl-β-cyclodextrin (HPβCD) as a complexation agent, a new class of DES was developed to combine the two solubilization-enhancing approaches induced by supramolecular complexation and the solvency capabilities of DES, generated using proper hydrogen bond acceptor (lactic acid, citric acid and choline chloride, ChCl).

The concept of combining CDs with DES has already been explored to merge the complexation capability of the former with the latter's solvent characteristics [3]. Different methods have been developed for this purpose and can be divided into two main categories: using CDs in an already prepared DES [4], or making CDs a component of DES itself, generating a new system defined as a supramolecular deep eutectic solvent (SUPRADES) [5].

Cyclodextrin-based supramolecular DES (CycloDES) were physical-chemical characterized and their potentiality as a drug vehicle were evaluated with three different BCS class II model drugs, specifically Cannabidiol, Indomethacin, and Dexamethasone, assessing the influence of different factors on the observed solubility and permeation compared with the only HPβCD/drug complexation.

As a result, CycloDESs emerged as a novel perspective strategy for addressing BCS class II and IV solubility issues, displaying at least a 100-fold improvement in the tested drug solubilities if compared to their water solubility. The use of different hydrogen bond acceptors allowed to choose the most suitable mixture for the specific model drugs and with the proper rheological behavior for further manipulations. Furthermore, when compared to a glucose-choline chloride DES employed as a reference, CycloDESs demonstrated to overcome one of the main soft spots of DES showing enhanced resistance to dilution. In fact, CycloDES kept a high proportion of drug in solution (i.e. 93% against 12% for Indomethacin), guaranteeing the proper concentration of solubilized drug in the adsorption site.

Among the newly introduced, ChCl:HPβCD CycloDES solubilized directly all the solid-state active principle ingredients without any extraction step from an already drug-loaded liquid media likewise oils.

The data from DSC, TGA, and NMR guarantee the proper solubilization and no degradation of the model drugs in the ChCl:HPβCD mixture.

The observed increased drug solubilization could reduce the use of CDs in the formulation, decreasing the overall cost of the preparations and possible cyclodextrins related side effects.

These results validate the solubility-enhancing effect, which is the key factor in delivering BCS class II and IV drugs, passing from solid source materials to favorable liquid vehicles, resulting also in a useful tool for the delivery of pH-sensitive drugs (i.e. CBD).

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POSTER N. 54

CHARACTERIZATION OF *H. CRENULATA* EXTRACTS AND QUANTIFICATION OF MARMESIN

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Introduction: *H. Crenulata* or thanaka is a tropical plant, commonly used by burmese people for making a paste used for skincare, especially for sun protection thanks to the presence of a dihydrocuranofumarin, named marmesin, with chromophore groups that allow the absorption of solar energy. This plant attracted the interest of cosmetic industries, but its chemical and biological properties are still mostly unknown [1]. The aim of this work was the full characterization of thanaka's extracts obtained from different sustainable methods of extraction and the potential application of these extracts for cosmetic and dermatological formulations.

Methods: Ten extraction protocols were applied by Soxhlet Extraction (SE) and Ultrasound-Assisted Extraction (UAE) methods. Thanaka bark was ground into a powder and dried at 35°C for 24 h. Five extraction solvents were used: water: ethanol mixtures (28% vol., 67% vol., and 48% vol.), a mixture of ethanol and ethyl acetate 50:50 (V/V) and demineralized water. For the UAEs, the samples were sonicated in an ice bath at a frequency of 20 kHz for 5 minutes and with an amplitude of 95% of the total energy of the sonicator (500 W). For SEs, the parameters (heat level and extraction cycles) of the extractor were changed based on the solvent that was used for each extraction. Solvents were removed under vacuum and the extracts were lyophilized and characterized for yield, antioxidant capacity (DPPH, ABTS, FRAP assays), Total Phenol Content (Folin- Ciocalteu assay) and anti-collagenase activity. Quantification of marmesin in the extracts was made with HPLC-DAD [2]. Human gingival fibroblasts (HGFs) cell line was used for cell proliferation assay, collagen release, and prostaglandin E2 release.

Results: The best extraction solvents in terms of antioxidant activity, phenol content and anti-collagenase activity were the hydroalcoholic solutions with 48% vol. and 67% vol., both for UAE and SE. The same extracts gave the best results also with the cell proliferation assay, the release of collagen and anti-inflammatory activity in HGFs. Comparing the quantity of marmesin found in these extracts, the one with the higher amount was the extract obtained with SE using the hydroalcoholic solution 67% vol.

Conclusion: The extractions allowed to obtain a powder with antioxidant and anti-collagenase activity, and with the potential to protect against UV light thanks to the presence of marmesin. The powder can be used for the formulation of an anti-age and sun protective face cream.

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POSTER N. 55

CARVACROL LOADED HYALURONIC-ACID COATED PLGA-NANOPARTICLES FOR ANTI-INFLAMMATORY AND ANTINOCICEPTIVE ACTIVITY

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Millions of people suffer from chronic pain induced by nerve injury, and it has been challenging to target and understand the neuronal activity responsible for this pain. However, the nervous and immune systems go hand in hand in causing neuropathic pain. The immune system's first response is to induce inflammation at the site of injury, and macrophages have been recognised to play an important role in the subsequent modulation of neuropathic pain. Hyaluronic acid (HA) is a well-known binder for the CD44 receptor on classically activated M1-macrophages. This targeted approach of HA can be used with the biocompatibility of poly (lactic-co-glycolic acid) (PLGA) to encapsulate carvacrol that interrupts the TNF-α pathway to negatively modulate pain and inflammation (Figure 1). Single emulsion solvent evaporation method is used to synthesize PLGA nanoparticles with cetyltrimethylammonium bromide (CTAB) as a surfactant. Charge interaction is used for the binding of HA and PLGA with assistance from CTAB [1, 2]. Successful coating of HA on PLGA-CTAB particles is determined by the final charge and the nanosize is optimised for effective macrophageal uptake. Particle size before and after coating with 1.5% HA was found to be 162 nm and 285 nm, respectively. The zeta potential for PLGA-CTAB particles was found to be 57.7 mV and for PLGA-CTAB-HA, it was -25.5 mV. The presence of a negative zeta potential indicated the successful coating of HA on PLGA-CTAB particles. Carvacrol-loaded PLGA-HA nanoparticles had a size of 225 nm. The SEM analysis of these nanoparticles indicated the size of 176 nm. The 4-month stability tests performed with DLS (201 nm) and zeta potential (-30.2 mV) for unloaded HA-PLGA nanoparticles showed a lack of agglomeration. Gas chromatography showed carvacrol encapsulation efficiency of 93.88% and a loading capacity of 18.78%. For the release studies, the solubility of carvacrol was tested in different media (1.4 mg carvacrol/mL), also mimicking the *in vivo* inflammatory environment. *In vitro* studies in presence of M1 and M2 macrophages were performed to evaluate the cell interaction of the delivery system.

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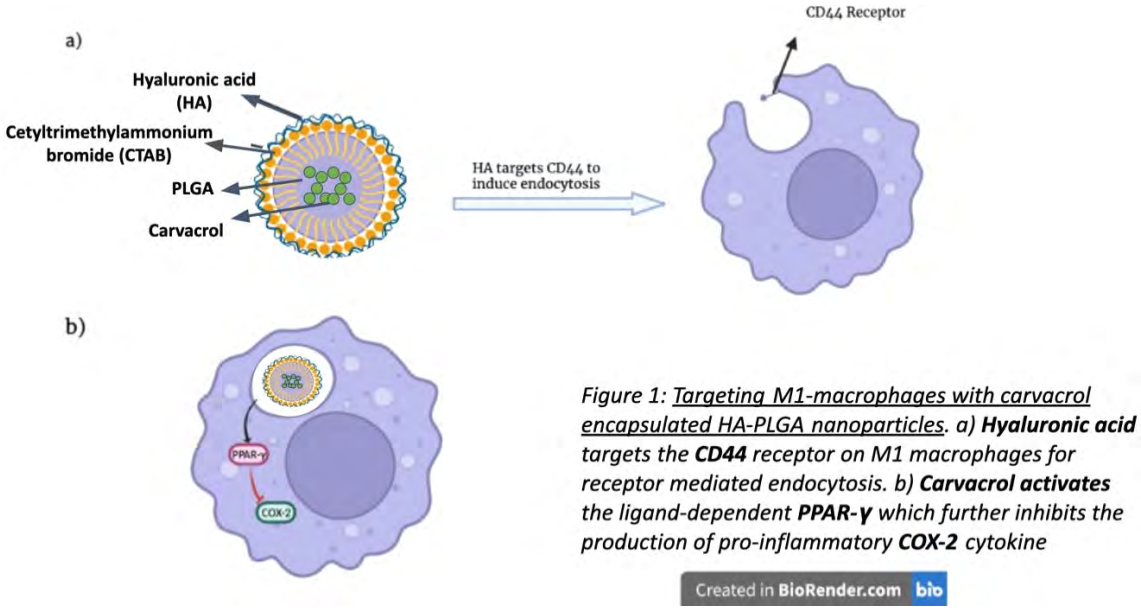

*Figure 1: Targeting M1-macrophages with carvacrol encapsulated HA-PLGA nanoparticles. a) **Hyaluronic acid** targets the **CD44** receptor on M1 macrophages for receptor mediated endocytosis. b) **Carvacrol activates** the ligand-dependent **PPAR-γ** which further inhibits the production of pro-inflammatory **COX-2** cytokine*

POSTER N. 56

EVALUATION OF MALEIC-PULLULAN/COLLAGEN FILMS FOR DERMAL APPLICATION

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Pullulan is a homopolysaccharide derivedfrom Aureobasidium pullulans, a polymorphic fungus. Its structure is composed of maltotriose units that are bonded to one another by α-1,6-glycosidic linkages [1]. According to its features, such as high solubility in water, less toxicity, good biodegradability, and non-immunogenicity, it is in widespread use in the pharmaceutical industry, like cosmetic industries, and in biomedical applications [2]. This work reports the development of a film formulation based on covalent crosslinking between maleic-pullulan and collagen to mimic extracellular matrix composition because of dermal application.

Pullulan was functionalized with maleic anhydride in DMSO to produce maleic-pullulan (MA-P) [3] and ^{1}H NMR spectra indicate 16.3% degree of modification. The obtained functionalized pullulan was crosslinked with collagen using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and N-hydroxysuccinimide to produce a viscous solution [4]. The film was obtained by dehydration at 37°C for 2 h and subsequently washed with milliQ until pH=7. ATR FT-IR confirmed the occurred reaction between the compounds, showing a difference in the amide group region (1550-1640 cm^{-1}). The mechanical property of the film was acquired showing a G'>G'' (G'=600 rad/s; G''= 100 rad/s) and a viscosity decrease with increased shear rate.

The biological evaluation was performed with McCoy mouse fibroblast cell line and bEND5 mouse brain endothelial cell line. Qualitative Live-Dead assay on McCoy cell line and quantitative PrestoBlue™ assay at 3,5 and 7 days on McCoy and bEND5 were performed to evaluate the cytocompatibility of the film and the data obtained confirm that material does not interfere with cell viability. In particular, the fluorescence intensity of bEND5 treated is higher than the control, suggesting good angiogenic properties. Fluorescenceimaging was performed and images showed that film does not lead to any change in the morphology of McCoy cells. Wound healing assay on McCoy cells was performed to evaluate the capacity of the film to promote cell migration and the images acquired at different time points showed good cell migration and complete scratch closure after 48 h. Wound healing assay on bEND5 is ongoing. PCR analysis was performed to evaluate the capacity of the film to stimulate the gene expression of healing promoters.Interestingly, the expression of *Vwf* gene (von Willebrand Factor), implicated in the biochemical cascade of coagulation, was mainly improved in bEND5 cultures. Additional investigation of the stimulated angiogenesis effect for the film-hydrogel matrix treatment is ongoing.

SEM images of freeze-dried film and cell cultured films (McCoy cell line) were acquired. The imagesconfirmed the good shape and wide spreadability of thecells on the film layer, confirming also the positive viability results of Live-Dead and PrestoBlue™ assays.The data suggest that hydrogel performance is excellent for dermal applications, allowing cell proliferation and migration. Moreover, cell viability on bEND5 and PCR analysis shows a high capacity of hydrogel to improve angiogenesis, further strengthening its possible application in dermal wound repair.

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POSTER N. 57

CBD LOADED TRANSFEROSOMES: DEVELOPMENT, EVALUATION, STABILITY AND *IN VITRO* PRELIMINAR STUDIES ON SCHIZOSACCHAROMYCES POMBE

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Cannabidiol (CBD) is a main non-psychoactive natural constituent of *Cannabis sativa* L. It has pleiotropic activity and it is used in many therapeutic treatment for multiple conditions [1]. Studies reporting pharmacokinetic data of CBD in humans have evidenced a scarce bioavailability after oral administration. Currently, oral CBD conventional formulations are challenging because of unpredictable release and absorption. Rational design of CBD loaded nanodelivery systems can provide a practical and ideal alternative formulation [2]. In the present study CBD was nanoencapsulated in transferosomes containing

increasing amount of Tween 20, chosen as edge activator, in order to increase both encapsulation efficiency and deformability. Deformable nanoliposomes were made of Phospholipoid 90G, cholesterol and Tween20, and exhibited ideal physical characteristics in terms of size (147.6±1.2 nm),

polydispersity index (0.193±0.070) and Z-potential (-45.81±1.43 mV). Transferosome shape and dimensions were confirmed using Trasmission Electron Microscopy (Figure 1).

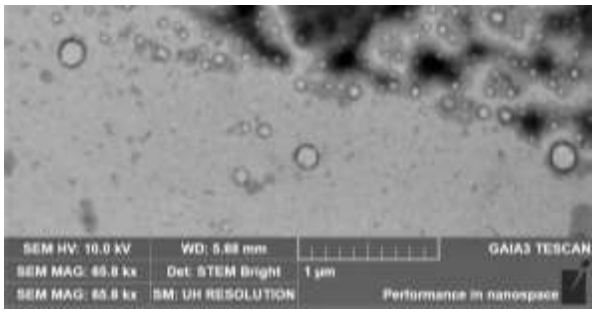

Figure 1. Picture of CBD-loaded transferosomes (Scanning and Trasmission Electron Microscopy)

Moreover, high percentage recovery and encapsulation efficiency, 99.01±1.41% and 90.55±1.22% respectively, were found using HPLC technique. Finally, the antigenotoxic potential of CBD both unformulated and loaded in liposomes (LP-CBD) was evaluated in *Schizosaccharomyces pombe* yeast model, using camptothecin (CPT) as a toxicity-inducing agent. The fission yeast *Schizosaccharomyces pombe* has become an increasingly model organism for studies concerning eukaryotic cells, to investigate various molecular and cellular processes [3].
First a spot assay was performed to ensure viability of yeast cells in presence of both CBD formulated and unformulated and empty liposomes and compare the effects of CPT after different co-administration.
By observing fluorescence microscopy, using DAPI as fluorescence dye, cells treated with CPT revealed some alterations, in particular an abnormal elongation when compared to negative control. CPT co-treated cells using both, individually, CBD and LP-CBD evidenced a decrease cell size, suggesting their potential in mitigating the damage caused by CPT.

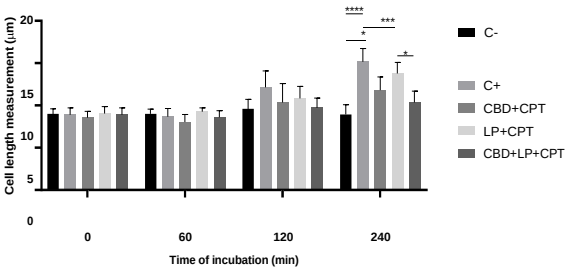

Figure 2 Graphic representation of the cell length measurement (µm) of the yeast S. pombe cells when exposed to 50 µg/ml CPT and/or 0.75 mg/mL of CBD, LP or CBD+LP samples in an incubation time of 0, 60, 120 and 240 minutes.

Even if the difference between CBD and CBD-LP was noticeable, evidence of different results came from cell cycle analysis. After ninety minutes, only the treatment containing CBD-LP showed the same results of negative control. These interesting results could be interpreted by the increased permeation of the formulated CBD when compared with the pure CBD.

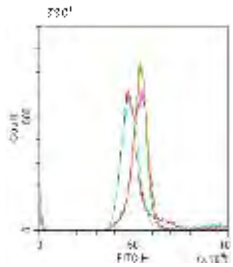

Figure 3. Cell cycle progression of *S. pombe* cells exposed to CPT and in the presence and in the absence (green) of CBD (violet), LP (orange) or LP-CBD (light blue) extracts by flow cytometry (negative control = red)

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POSTER N. 58

CHARACTERIZATION OF KETOPROFEN, LYSINE AND GABAPENTIN CO-CRYSTAL: PREFORMULATION STUDIES OF A NEW CHEMICAL ENTITY

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Ketoprofen, lysine salt (KLS) and gabapentin were chemically combined via co-crystallization to yield a ternary drug-drug co-crystal of ketoprofen, lysine and gabapentin (KLS-GABA co-crystal). Using relevant *in vitro* and *in vivo* models, it was demonstrated that KLS-GABA co-crystal have supra- additive effects in modulating key pathways in neuropathic pain and in gastric mucosal damagecompared to KLS alone or in combination with gabapentin. Considering the important therapeutic effects, KLS-GABA co-crystal was proposed as a new drug candidate with high potential clinical benefit–to– risk ratio for chronic pain treatment [1]. A detailed characterization of KLS-GABA co-crystal, as solid state, was carried out investigating the physical,technological and analytical properties with the aim to realize an oral solid formulation. The attention waspoint out toward powder morphology, analysed using a Scanning Electron Microscope (SEM Pro-X),flowability analysed through Flodex apparatus and tapped and bulk density, the particle size distribution (PSD) using a granulometer (Mastersizer 3000 – Malvern) and by sieves analysis. The powder showed aroughly round shape, with a coarse surface, and a PSD characterized by a D_{10}: 51.55 µm D_{50}: 266.59 µm and D_{90}: 479.92 µm. The Hausner ratio, the compressibility index and Flodex showed a good flowability of the powder. The new solid phase was also analysed by thermal analyses: differential scanning calorimetry (DSC), thermogravimetric analysis (TGA) were carried out using Perkin Elmer equipment. The thermogram showed a single well-defined melting point peak characteristic of the crystalline structure of the substance, while TGA thermogram showed a weight loss of 0.132% (in the range of 30°C and 120°C). FT- IR analysis was also performed: the spectrum of KLS-Gabapentin shows the characteristic peaks of KLS and gabapentin.

Drug-excipient compatibility studies were then performed with the intent to identify, quantify any potential interactions (physical or chemical) affecting the manufacturability, quality and performance of the final drug product. [2] Binary mixtures of drug and single excipient were mechanically stressed involving procedures as grinding using a ball mill. The purity profile of the KLS-GABA co-crystal was monitored through different analytical technics [3].

All the collected data will drive in choosing the most useful excipients for the final oral solid formulation. A DoE (Design of Experiment) will be implemented to define the quality and quantitative composition in relationship with the product quality attributes of such as hardness, disintegration time and release profiles. [4]

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POSTER N. 59

CELLULOLYTIC ENZYMES IN HIGH-VISCOSITY HPMC COATINGS FOR A TIME-DEPENDENT ORAL COLON DELIVERY SYSTEM: APPLICATION BY SPRAY-COATING

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Introduction

Hydroxypropyl methylcellulose (HPMC)-coated oral delivery systems have been investigated forpulsatile release and, when provided with a gastroresistant outer film, for time-dependent colonic release. The HPMC layer applied to a drug-containing core, such as a tablet or a capsule, swells and erodes upon contact with the aqueous media, thus being responsible for a lag phase prior to a desirable prompt and quantitative drug release[1]. Among the techniques available for the polymer deposition, spray-coating generally allows a homogeneous layer to be formed that can be easily modulated in thickness. Methocel® K4M, a high-viscosity HPMC grade previously evaluated as a coating agent for these delivery systems,showed superior ability to defer the onset of release compared to lower viscosity HPMC, *i.e.* Methocel®E50 and Methocel® E5[2]. Indeed, a thinner Methocel® K4M layer was required to obtain a unitary *in vitro* lag time. However, when systems coated with Methocel® K4M to 20% weight gain were tested *in vivo*, the absorption of a tracer drug (acetaminophen) after the lag phase turned out to be undesirably slow. This was ascribed to release being hindered by a persistent gel layer the drug had to diffuse through to reach the luminal fluids[3].

To address such a drawback, the addition of acellulolytic enzymatic complex (cellulase) into the HPMC layer was proposed. Hydrolyzing β-1,4 glycosidic bonds, cellulase could cleave the polymer into shorter length chains, which would result in a progressively weaker, more erosive gel layer, exertinga less persistent barrier effect around the drug-containing core[4].

The incorporation of this enzymatic complex, used in the form of a commercially available powder product (Sterzym® C13030), was proved advantageous in a previous work, where Methocel® K4M was applied onto 4 mm tablet cores by press-coating technique[3].

Based on the above premises, the purpose of the present work was to evaluate the feasibility of this approach when the polymeric layer was applied by aqueous spray-coating, which would allow for a higher flexibility in the modulation of the lag phase duration.

Methods

Immediate-release convex tablet cores (Ø 4 mm, nominal weight 40 mg) containing 80% of paracetamol were manufactured by a rotary tablet press (AM-8S, Officine Ronchi, Italy) and coated in a bottom-spray fluid-bed equipment (Mini-Glatt, Glatt GmbH, Germany) with a 2% w/V Methocel® K4M water solution, either alone or alternated with a 10% w/V solution of a cellulolytic product (Sternzym® C13030). The coated systems were checked for coating thickness by a digital caliper. SEM photomicrographs of cross-sectioned units were acquired after gold-sputtering at 450x magnification. The HPMC-coated systems were enteric coated by the same equipment with a 57% w/w Eudragit® L30D-55 aqueous dispersion containing glyceryl monostearate, triethyl citrate and Tween® 80. Release tests were performed in a USP 43 disintegration apparatus (DT3 Sotax, Switzerland)using 800 mL of compendial fluids. These were pH 6.8phosphate buffer either alone or preceded by pH 1.2 buffer for 120 min (37±1 °C, 100 rpm, λ = 243 nm).

Results

The rapid hydrolysis of HPMC occurring in the presence of cellulase, as assessed by viscosity measurements (data not shown), ruled out the possibility of using enzyme/polymer co-solutions as coating formulations. Thus, the relevant deposition in distinct phases was necessary, leading to a configuration of the coating where HPMC and cellulase were confined to separate, alternate layers (Figure 1). In particular, to evaluate this design concept, a multi-layer system was manufactured containing 10% w/w of enzyme product on the total amount of HMPC. Sternzym® C13030 was applied in 4 thin layers of approximately 10 μm in thickness alternating with 100 μm thick HPMC layers, to an overall 500 μm thickness of the coating (S_5L).

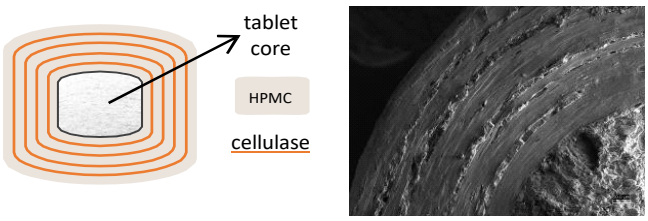

Figure 1: diagram and SEM photomicrograph of a cross-sectioned system coated with Methocel® K4M and Sterzym® C13030

For comparison purposes, systems having HPMC coating of same nominal thickness without enzyme were also prepared (R_500).
The release profiles of the coated units are reported in Figure 2.

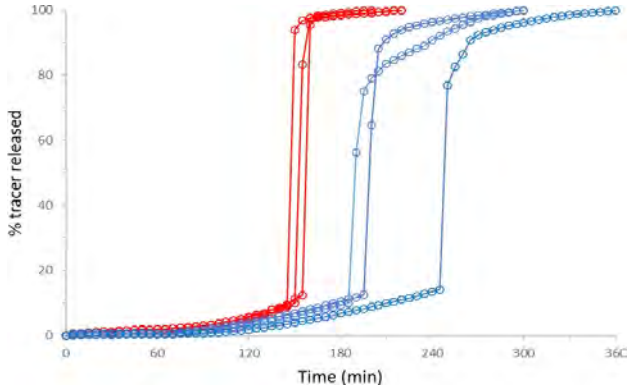

Figure 2: release profiles from R_500 (blue) and S_5L (red) in phosphate buffer pH 6.8 (n=3).

In the absence of Sternzym® C13030, the average lag time, *i.e.* the time to breakup of the system corresponding to a steep increase in the release rate, was of 208 min ± 32 min. The release profiles were characterized by diffusional release largely coveringthe lag phase, up to approximately 15% of the drug content. After breakup, coinciding with fast release of most drug content, a slower release phase occurred,possibly due to persistence of a gel layer around the core. This particular release pattern would be in agreement with the aforementioned pharmacokinetic results[3]. The use of the enzyme resulted in a higher reproducibility and clear reduction of the lag time (150 min ± 5 min). A certain ability to counteract the slow release effect associated with the enduring gel layer after the core disintegration was also noticed.
Finally, the coated units either with or without cellulase were provided with an outer gastroresistant film (S_5L_GR and R_500_GR) to attain the colonic release configuration.
These formulations were evaluated for releaseaccording to the two-stage testing method (Figure 3).

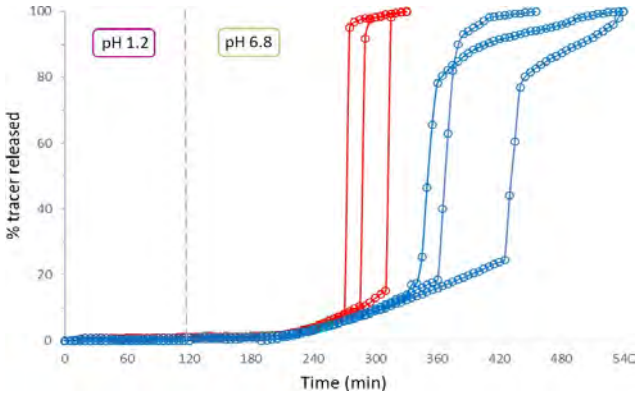

Figure 3: Release profiles from R_500_GR (blue) and S_5L_GR (red) in hydrochloric acid pH 1.2 (2h) and phosphate buffer pH 6.8 (*n*=3).

Gatroresistant formulations with and without cellulase were shown to withstand the acidic stage of the test. In the phosphate buffer medium, mean lag times of 168 and 255 min, respectively, were obtained, *i.e.* slightly longer compared to the corresponding non- gastroresistant systems. This would be consistent with the time taken for the enteric coating to dissolve after the pH change.

Conclusion

The incorporation of a cellulolytic enzyme into the high viscosity HPMC-based functional layer of a time-dependent delivery system obtained by spray-coating was demonstrated to be effective in improving the release performance as compared with the original configuration without enzyme. The previously observed issues of diffusional release and, particularly, of poor reproducibility as well as flexibility of the lag phase duration were indeed addressed.

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POSTER N. 60

ICOS-Fc TARGETED ALBUMIN-BASED NANOPARTICLES AS A TOOL FOR THE DELIVERY OF DOXORUBICIN IN OSTEOSARCOMA CELL LINES

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Albumin-based nanoparticles (NPs) have emerged as a versatile platform for the efficient and targeted delivery of diverse chemotherapeutic agents, owing to their biocompatibility, safety, and ability to easily modify their surfaces for specific targeting purposes. Doxorubicin is an antineoplastic agent utilized for treating a broad spectrum of cancers. Nevertheless, it has several limitations, including dose- dependent cardiotoxicity, a lack of selectivity for tumour cells, and the development of cell resistance.
[1] Although a liposomal doxorubicin nanoformulation (Doxil®) is available on the market, extensive research continues to concentrate on designing nanocarriers for targeted doxorubicin delivery to tumour cells.[2] The aim of the work was to develop nanoformulation to improve doxorubicin delivery and overcome cancer resistance. In this work, the preparation of albumin-based nanoparticles (NPs) was accomplished by using an ultrasonication technique. The research involves the development of bovine serum albumin-based doxorubicin-loaded NPs (BSA-DOX NPs) and conjugating them with ICOS-Fc. ICOS-Fc is an inducible costimulatory molecule expressed mainly on activated CD4+ T-cells activation. Bovine serum albumin (BSA-NPs) was obtained by a suitable tuned double-emulsion method. Doxorubicin Hydrochloride (DOX-HCL) was dissolved in aqueous Span83 and N-methyl pyrrolidone solution in a 70:30 ratio v/v. This solution was placed in a sonication bath for 10 minutes. DOX-HCL solution was added to a Miglyol 829 and sonicated by using an ultrasonication technique (20KH,500W) to form a water-in-oil emulsion. Then a albumin solution is prepared in a phosphate buffer atpH 8.0 containing Kolliphor HS15. Subsequently, the water in the oil emulsion was dropwise added to the albumin solution. The system was sonicated again to obtain BSA-DOX NPs. The BSA-DOX NPs was purified by dialysis against a saline solution. The second formulations formed Blank-BSA NPs and BSA-DOX NPs are conjugated with ICOS-Fc. Albumin NPs, conjugated with ICOS-Fc, blank NPs and BSA-DOX loaded NPs were characterized by determining their physicochemical parameters (i.e., average diameter, polydispersity index and zeta potential). The morphology of the nanoparticles was analyzed using scanning electron microscopy (SEM). Moreover, doxorubicin loading capacity, encapsulation efficiency, *in vitro* release kinetics, and *in vitro* physical stability over time were evaluated. Thehaemolytic activity of all the type NPs was evaluated in mice blood. Biological assays were performed on HOS and U2OS cell lines obtained from the white bone of a 13-year-old female patient diagnosed with osteosarcoma. MG-63 cells, which exhibit fibroblast morphology, were isolated from the white bone of a 15-year-old male patient with osteosarcoma and demonstrated resistance to doxorubicin. All the type NPs demonstrated an average diameter of about 300 nm and a negatively surface charged with a zeta potential of about -30 mV. Furthermore, the NPs exhibited a favourable drug loading capability (1.58%) and good encapsulation efficiency (95%). After 24 hours, it was observed that the cumulative percentage of doxorubicin release from the NPs was about 26%, indicating the NPs ability to sustain the release of the drug over an extended period. The physical stability of all the types of NPs, including their size, zeta potential, and morphology, remained consistent for a period ofsix months when stored at 4°C. Additionally, all the types of NPs remained stable under physiological conditions at 37°C in the presence of Seronorm HumanPlasma. Moreover, no significant haemolytic activity was observed. The biological assays conducted on HOS, U2OS, and MG-63 cell lines revealed good viability and demonstrated promising results. Furthermore, osteosarcoma cell lines displayed a positive response to the BSA-DOX NPs conjugatedwith ICOS-Fc, indicating enhanced efficacy in overcoming resistance.
In conclusion, the development of BSA-DOX NPs conjugated with ICOS-Fc shows promise for improving drug delivery and overcoming resistance in cancer treatment. The NPs demonstrated favourable physicochemical properties, sustained drug release, and good stability over time. Biological assays on osteosarcoma cell lines exhibited positive results, indicating enhanced efficacy. These findings highlight the potential of this nanoformulation as a targeted and efficient approach to osteosarcoma cancer therapy.

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POSTER N. 61

Investigation of taste-masking effectiveness of Eudragit® E PO as coating agent for oregano essential oil tablets

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Oregano essential oil (OEO) has antioxidant, antiproliferative and anti-inflammatory activities. However, its oral use is strongly hampered by its irritating taste and high sensitivity to light and humidity. Recently, numerous reports have described various strategies for essential oil encapsulation including the preparation of micro- and nanoparticles and the formation of inclusion complexes with cyclodextrins [1], but because the procedures are often laborious and time-consuming, an advantageous alternative is represented by solid dosage forms such astablets and capsules. These forms are easily administered leading to better compliance and offering protection against light and humidity. OEO is already marketed as a diet supplement for humans, mainly as soft gelatine capsules but the production costs are considerable. Thus, the aim of this work was to develop and characterize tablets based on zeolite granules containing OEO and coated with Eudragit® EPO to protect the oil from instability and simultaneously cover up its flavour and aroma. Because the zeolite has a large surface area that facilitate the absorption of essential oil [2] zeolite was used as absorbent material for OEO in the ratio zeolite:OEO 20:1. The obtained dried zeolite containing OEO was mixed with hydroxipropilmethylcellulose (HPMC) in the ratiozeolite:HPMC 7:3 by adding 400 µL of water. Theresulting moisture was extruded to form granules of 1.2mm size. Subsequently, granules were compressed into flat-faced tablets of 0.6 mm diameter and 20 mg weight, with a hydraulic press, applying a force of 1000 kg for 15 seconds, to obtain the OEO core tablets.The OEO cores were compression-coated with Eudragit® EP O maintaining core:coat weight ratio at 1:2 [3]. Briefly, the tablets were prepared by first filling one half (20 mg) of the coat into the die cavity, then positioning the core centrally and finally filling the remaining half of the coat (20 mg) on the top to be directly compressed, using the parameters described above. The coated tablets consisted of 13.4 mg of zeolite, 0.6 mg of OEO, 6 mg di HPMC and 40 mg of Eudragit® EPO. Each coated tablet was subjected to the thermogravimetric analysis, disintegration, resistance to crushing as reported in the European Pharmacopoeia 10th edition, release study and cell viability studies *in vitro*. Thermogravimetric analysis demonstrated that the OEO was effectively protected from degradation and irritating taste by coated tablets. Indeed, in the thermogravimetric curve of core tablets there was a weight loss at 154 ºC ascribable to OEO, that is not present in the thermogravimetric curve of coated tablets. This could be due to the effect of the Eudragit® EPO coating, that stabilizes the OEO, moving its volatilization at higher temperature (250- 450°C) where all the other tablet components decompose. The effectiveness of the coating was demonstrated also by the disintegration and resistanceto crushing tests. The core tablets disintegrated in waterat 37±2 ºC within 15 minutes; the coated tablets disaggregated in HCl 0.1M at 37±2 ºC within 30 minutes. The resistance to crushing performed using Monsanto hardness tester confirmed that only the coated tablets had sufficient mechanical strength (measured force 4N). Regarding the release of OEO, as expected, the coating did not affect the release of OEO from the tablets. Indeed, the release appeared tobe controlled by the matrix erosion process of HPMC [4]. Preliminary biological evaluations on human colorectal adenocarcinoma Caco-2 cells wereperformed and the *in vitro* cell viability assay showed that OEO was non-cytotoxic in the range 3-50 µg/ml. Biological investigations in terms of OEO antioxidant activity in the formulations are ongoing.

In conclusion, Eudragit® E PO coated tablets represent a powerful tool for the oral administration of essential oil derived from plants, protecting the essential oil from degradation and simultaneously providing taste-masking of unpleasant odour and flavour.

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POSTER N. 62

Camouflaged nanosystems: an innovative strategy to overcome blood brain barrier (BBB) resistance in neurodegeneration.

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The blood–brain barrier (BBB) is a highly selective semipermeable border of endothelial cells andrepresents the main protection of the Central Nervous System (CNS). It plays a critical role in controlling the influx and efflux of biological substances, between the blood and the brain. BBB function is strongly regulatedby the presence of tight junctions and by different types of active transporters. Among them, P-glycoprotein (P- gp) is widely expressed in the apical side of endothelialcells [1]. P-gp is a transmembrane glycoprotein,particularly located in intestine and brain, which regulates the efflux of structurally heterogeneous drug, including Donepezil, an acetylcholinesterase inhibitor currently used to treat dementia in Alzheimer Disease patients [2].
Thus, the specific targeting of BBB coupled with P-gp inhibition could potentiate CNS delivery of neurodegenerative treatments. For instance, camouflaged nanocarriers, consisting in a drug loaded core surrounded by cell derived membranes or lipids mixture biomimicking cell surfaces, could be a potential tool to guide drug delivery towards the brain.
As a first step, a nanoparticle (NP) prototype was developed, formed by a central core made of depolymerized chitosan surrounded by lipid shell consisting of either synthetic lipids (NP-L) or cell membrane (NP-M) extracted from bEnd.3 murine endothelial cell line. The prepared NP showed a size diameter of 150 nm. The cytoplasmic membrane isolation was carried out and immunostaining studies confirmed the maintenance of cell adhesion molecules within the collected membranes (vascular cell adhesion molecules-1, V-CAM 1, vascular endothelial cadherin, VE-cadherin). NP were coated by thermal controlled co-extrusion, resulting in size increment and reduction of the Zeta potential values for both NP-L and NP-M. The coating was confirmed by immunostaining studies and cytotoxicity evaluation was also performed. Coated and uncoated fluoresceinated nanoparticles underwent to BBB *in vitro* permeation study, by using bEnd.3 monolayer cell model. The study confirmed that NP-M were internalized to a higher extent with respect to NP and NP-L with the maximum internalization at 1 hour. However, after 3 hours of incubation, the analysis indicated a decrease in nanoparticles uptake, probably due to P-gp activity. In this perspective, once verified bEnd.3cells targeting, the second step of this project was the development of new Donepezil-loaded liposomes containing in the external lipidic membrane SG44, a P-gp inhibitor (SG44, IC_{50}= 7.1nM, compound10 in Nesi et al [3]). coated with bEnd.3 cell derived membranes. Three liposomal formulations composed by different lipids ratio: 1. DPPC:DOPC:Chol (6:3:1 w/w), 2. HSPC:DMG-PEG:Chol (3:1:1 w/w) and
3. DPPC:DOPC:DMG-PEG:Chol (5:2:2:1 w/w) were prepared by film rehydration and extrusion sizing. Liposomes of average diameter in the range 100- 160nm were obtained. Dual drug loading was performed, dissolving SG44 in the lipidic film and Donepezil in the aqueous phase, with a drug loading from 45% to 90%. Future investigations on camouflaged Donepezil/SG44 loaded liposomes will clarify whether our delivery system could increase Donepezil brain uptake/bioavailability through the inhibition of P-gp mediated drug and nanoparticle efflux.

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POSTER N. 63

From extraction to formulation: development of plant extract-based natural products

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Plant matrices, rich in healthy and bioactive natural compounds, have long been a primary source to produce medicine, cosmetics, and food supplements. Most of the extraction processes rely massively on solvents, the majority of which have petroleum origins. The ideal alternative solvents suitable for green extraction should have high solvency, high flash points with low toxicity and low environmental impacts, be biodegradable and obtained from renewable resources at a reasonable price and be easy to recycle without any deleterious effect on the environment.

The objective of this work was to investigate the ability of the complexation agents cyclodextrins (CDs) and two deep eutectic solvents (DESs) to extract bioactive molecules from two plant matrices.

For this purpose, *Cynara scolymus* (CS) and *Crithmum maritimum* (CM) were chosen as model plant matrices to test these novel extraction methods.

CS, usually known as artichoke, is a Mediterranean plant with lipid-lowering and antioxidant properties dueto its content of polyphenols, including caffeoylquinic and dicaffeoylquinic acids [1]. On the other hand, CM, also known as sea fennel, has lots of beneficial health effects, is rich in antioxidants, and, more recently, it is also appreciated for its anti-cancer properties against hepatocellular carcinoma (HCC) [2].

A preliminary study of the literature highlighted that both plant matrices have a similar polar fraction content, constituted particularly by chlorogenic acid (CA) and its derivatives. While CS apolar fraction is not considered interesting from a nutraceutical and healthy point of view, CM apolar fraction was found to be very appealing, because of its content in various substances classes responsible for its beneficial effect against HCC.

Starting from these assumptions, a systematic extraction study was conducted on both plant matrices. Several parameters, such as temperature, extraction time and vegetable matrix/solvent ratio were considered in the extraction processes, using as solvents water containing both native and substituted CDs, like alpha, beta, gamma, and hydroxypropyl-beta-CD (HP-beta-CD), and a novel DES, called CycloDES, composed of HP-beta-CD and choline chloride.

According to their solubility properties, CDs were solubilized in water in a concentration range between 1 to 29.5 % w/V and a 40% w/w water containing CycloDES (CDES) was investigated. The liquid extracts obtained from the extraction processes were analyzed and characterized using a high-performance liquid chromatography with diode-array detection (HPLC-DAD), and titrated in total caffeoylquinic acids, expressed as CA (% w/V). For both CS and CM extracts, the results showed that the presence of low percentages of CDs in the extraction solvent provided significantly higher caffeoylquinic acids content compared to water, but less than 70° ethanol sample. On the other hand, the presence of high concentrations of HP-beta-CD and CDES significantly increased the content of the active substances compared with both water and 70° ethanol samples. Extracts obtained with CDs enriched water were also freeze-dried, obtaining a powder extract, while CDES extracts were not furthermore processed. Both solid and liquid extracts will be evaluated for their lipid-lowering, antioxidant, and anti-inflammatory properties.

Regarding CM apolar fraction, extraction studies showed that the ethyl acetate extract contained substances that show high efficacy in synergistic pharmacological therapies for the regression of HCC [2]. This evidence led to research and discover lipophilic DESs (LDES) with solvency abilities similar to ethyl acetate. In particular, a dodecanoic and octanoic acids-based DES was used for this purpose. Liquid extracts, which also involved the use of ultrasounds, obtained with the LDES were characterized using the HPLC technique too. Results showed that LDES extracts had an active substances content even higher than ethyl acetate extracts, indicating that this could be a viable alternative to the organic solvent. CM extracts are currently under test on HCC cellular lines, to assess their efficacy. Considering the obtained results, formulation studies will be carried out on the extracts aiming at the realization of pharmaceutical dosage forms that can increase the extract stability and maximize its absorption and bioavailability. Moreover, these techniques have provided an increase in extraction yields, but most importantly, the resulting extracts could be already used as nutraceutical supplements or could be considered the starting point to realize different dosage forms.

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POSTER N. 64

MUCOSAL APPLICATION OF NANOGEL FOR THE NASAL ADMINISTRATION OF ANTIGENS

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Nasal administration has been investigated in recent years as an effective mucosal site suitable for non-invasive vaccine delivery and able to elicit both systemic and mucosal immunity [1]. Delivery of proteinwithin the epithelial barrier is a key point to induce an immune response. In this framework, nanogel (nGEL) can be properly designed to present the delivered antigen to the immune system in controlled release formulations promoting their targeting to specific immune populations. Physico-chemical properties ofnGEL may be determining factors in designing the induced immune response dictating its ability to facilitate antigen transmucosal transport and its further access to the immunocompetent cells. The final featuresof nanogels (*i.e.*, mean size, surface charge) can be influenced by several variables such as the polymer concentration and type, the ratio between polymers and charges (critical material attributes) and different critical process parameters, such as ionic strength, temperature,and pH, used during their preparation. Taking into account these considerations, the selection of raw materials and the composition of such systems is very crucial and was studied according with Quality by Design (QbD) approach [2]. Herein, nGEL based on Dextran Sulfate (DS) and ?-Poly-lysine (εPL) were designed by the Response Surface Methodology (RSM) approach for nasal administration investigating the effect of critical independent variables on nGEL features. The nanosuspensions were prepared by polyelectrolyte complexation which is an eco-friendly method based on electrostatic interactions between polyelectrolytes of opposite charges [3]. The optimized formulation was successfully loaded with ovalbumin (OVA) and/or OVA-FITC, selected as model antigen, and characterized by a physical-chemical and technological point of view to evaluate mean size, polydispersity, stability, mucoadhesive strength, thermal behavior, antigen entrapment efficiency and in vitro release profile. Cytotoxicity analysis proved the safety of the optimized nGEL toward Human keratinocyte (NCTC 2544), human fibroblast (HFF1) and human airway epithelial (Calu-3) cell lines, selectedas skin, nasal and bronchial/tracheal airway epithelium cell-based in vitro models, respectively. Innate immune response was evaluated through the in vitro activation of human primary monocytes and monocyte-derived macrophages and the inflammatory induction was measured in terms of pro- and anti-inflammatory cytokine production (*i.e.*, TNF-α, IL-6, IL-10). Results showed that nGEL did not induce a reactivity in both monocytes and monocyte-derived macrophages when compared to LPS stimulation. In order to link innate and adaptive immune responses, murine Bone Marrow derived Dendritic Cells (BMDC) and $OVA_{(257-264)}$ specific T-Cell hybridoma were used to assess the antigen presentation ability. nGEL engulfed by BMDC are processed and the $OVA_{(257-264)}$ antigen is exposed in the MHCI (H2-Kb) groove, where is recognized by the B3Z hybridoma T Cell Receptor, inducing T cell activation and IL-2 production. While IL-2 production in response to soluble OVA is scarce, the antigen entrapment in nGEL lead to a significant antigen internalization and presentation, driving the strong activation of OVA specific T cells.

Moreover, uptake kinetics and intracellular trafficking of nGEL were investigated by CLSM in murine dendritic cells.

The results suggested that BMDC exhibited evident green fluorescence signals, demonstrating strong capability of nGEL in enhancing antigen uptake and after internalization, antigen is processed in autophagy-mediated intracellular trafficking for MHC class I cross-presentation. Finally, in order to deeply evaluate the potential application of nGEL for mucosal vaccine application, *in vivo* studies were performed on mice to track the antigen delivery in airway mucosa and its biodistribution after nasal administration. The obtained results demonstrated and increased residence time of OVA-FITC in the nasal mucosa after its encapsulation within nGEL.

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POSTER N. 65

MICROFLUIDIC PRODUCTION OF BIOMIMETIC LIPOSOMES FOR PERSONALIZED THERAPY OF METASTATIC MELANOMA

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Nanoparticles (NPs) modified by cell membranes represent an emerging biomimetic platform that can mimic the innate biological functions resulting from the various cell membranes in biological systems. Many research investigations have demonstrated the potential utility of biomimetic NPs in the treatment of cancer. As a simple and effective approach, delivery vehicles consisting of cell membranes are extensively researched and found to have various merits, such as prolonging the circulation time, alleviating immunogenicity, and accomplishing active targeting [1].

In this study, we investigated the use of microfluidic technology to produce biomimetic liposomes (hybrid liposomes) by fusing synthetic lipids directly with cell membranes (CM) obtained from a metastatic melanoma cell line (MM) extracted from a patient biopsy material. Here, a microfluidic sonication strategy for one-step and continuous generation of liposomes and hybrid liposomes is proposed to address the challenge of breaking the CM by purely hydrodynamic forces in microchannels. (Fig. 1). Two polypropylene microfluidic devices fabricated using 3D printing technology with different geometries were tested. Due to their complex internal structures, both geometries produced high-quality monodisperse hybrid liposomes by passively mixing the two phases containing lipids and CM, respectively. [2,3].

To evaluate the best hybridization conditions, we produced three hybrid liposomes formulations starting by three different amounts of CM. First, we demonstrated the effective fusion of the CM with liposomes through dynamic light scattering, nanoparticle tracking analysis, fluorescence resonance energy transfer (FRET) and flow cytometry characterizations. To explore the homotypic targeting strategy, 2D and 3D *in vitro* uptake studies were performed, showing that the hybrid liposomes had a stronger affinity for its source MM cancer cells than for hepatocellular carcinoma cancer cell line, with an 8- fold higher cellular uptake compared with liposomes. Moreover, to candidate this biomimetic nanosystem as a potential therapeutic tool for the personalized treatment of metastatic melanoma, cobimetinib and lenvatinib, were efficiently loaded, demonstrating an in vitro higher antitumor efficacy referred to the free drugs administration.

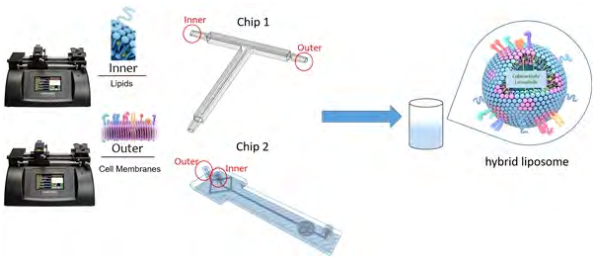

Figure 1. Schematic representation of setup producing hybrid liposome through microfluidics.

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POSTER N. 66

AZITHROMYCIN-LOADED VESICLES FOR THE TREATMENT OF SKIN INFECTIONS: A COMPARATIVE STUDY OF DIFFERENT FORMULATIONS

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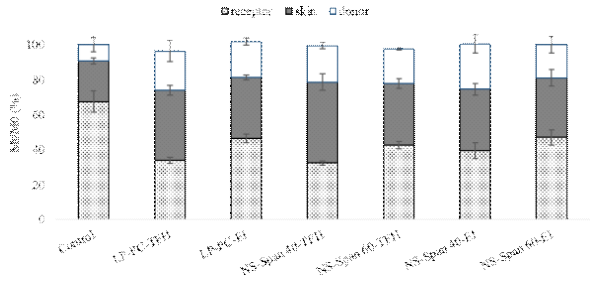
In recent years, the rise of bacterial skin infections together with the lack of adequate therapies have urged the scientific community to focus on the development of new and more efficient treatment strategies [1]. The administration of antibiotics through the topical route is the first-choice treatment for these infections. In fact, localized therapy improves drug safety profile and limits systemic exposure, responsible for the development of resistance [2]. However, limited drug skin retention can lead to an insufficient concentration of the active molecule at the site of action, which in turn requires multiple and frequent administrations, with a negative impact on patient compliance [3].

In this context, the development of new drug delivery systems able to improve topical therapy still remains an important challenge. Indeed, the aim of this study was to prepare niosomes (NS) and liposomes (LP), containing a broad-spectrum antibiotic such as azithromycin (AZT), for the treatment of bacterial skin infections.

NS (vesicles formed by the self-assembly of non-ionic surfactants) were prepared by employing sorbitan monopalmitate (Span 40) or sorbitan monostearate (Span 60), while LP (vesicles composed of phospholipid bilayer shells) were obtained with phosphatidylcholine from egg yolk (PC; 80.1% L-α- phosphatidylcholine) or from soybean lecithin (SL; not less than 94% L-α-phosphatidylcholine). Two widely used preparation methods have been chosen: 1) thin film hydration followed by extrusion (TFH) and 2) ethanol injection (EI). The formulations were then characterized for their size and polydispersity index (photon correlation spectroscopy-PCS; Brookhaven 90-PLUS instrument), zeta potential (Malvern Zetasizer3000 HS instrument), encapsulation efficiency (dialysismethod; Visking Tubo Dialysis membrane, Medicell International Ltd., 14,000 Da molecular weight cut- off), stability at room temperature (RT) and at 4-8 °C for 180 days (PCS), and ability to release the drug(Franz cell diffusion system, PermeGear; MF-Millipore® Membrane Filter, 0.22 µm pore size; T = 32
°C) and promote its retention inside the skin (Franz cell diffusion system; porcine ear skin; T = 32 °C). In addition, the antimicrobial activity toward the most representative pathogens (*S. aureus*, *C. acnes*) of the skin infections was tested using the broth dilution test. Finally, to determine the cytotoxicity, fibroblasts were exposed to the formulations and their metabolicactivity and viability were assessed by using the Resazurin reduction based-assay and by countingliving cells.

Vesicles prepared through TFH showed higher sizes with respect to the corresponding formulations obtained by the EI method. All the vesicles presented a low polydispersity index, an adequate encapsulation efficiency and a negative zeta potential, which ensured the stability of the vesicle suspensions during the storage period (except for liposomes composed of SL). Formulations prepared through TFH provided a sustained AZT release than the vesicles obtained by EI method, because of the smaller surface area. Moreover, all the vesicles increased drug retention inside the skin in comparison to the control.



The percentage amount of AZT in the receptor compartment, within the skin and in the donor compartment obtained after 24 hours from the application of control, LP or NS (means ± SD, n = 5).

In general, the lower was the released drug amount, the higher was the retention of AZT inside the skin. Among all the formulations, NS-Span 40-TFH and LP- PC-TFH guaranteed the retention of the greatestamount of AZT inside the skin. The latter capability could ensure the minimization of dosage and frequencyof administration and limit the risk of antibiotic drug overuse. Finally, NS-Span 40-TFH and LP-PC-TFH showed appreciable antimicrobial activity against the selected pathogens, and did not exhibit any cytotoxicity, thus representing promising nanocarriers able to improve the effectiveness of skin infection treatment.

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POSTER N. 67

PULMONARY ADMINISTRATION OF ENOXIMONE: FROM OFF LABEL ADMINISTRATION TO NEW DRY POWDER FORMULATION

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The lack of effective acute respiratory distress syndrome (ARDS) therapies was highlighted duringthe COVID-19 pandemic. The present work aims to confirm the clinical evidence[1] for pulmonary administration of Enoximone (phosphodiesterase 3 inhibitor). Firstly, improved clinical data and biopharmaceutical evaluations of the off-label use of an intravenous formulation (i.v.-ENOX) commercially available (PERFAN®) were collected and then the formulation of inhalable dry powders was assessed.

Clinical data were collected at the Hospital of Livorno from 14 patients (mean age 65±14) admitted to intensive care with moderate to severe CARDS and a PaO2/FiO2 =150 after oxygenation therapy or high flow oxygen ventilation or CPAP. Patients were treatedwith 50 mg i.v.-ENOX (via a Benefis jet nebuliser), twice a day for 48 h. Nine patients were responders, with increased PaO2/FiO2 after 48 h of aerosolisedtherapy (from 140 to 210, p = 0.01), reduced cough, dyspnoea and breaths/min. No serious side effects wereregistered. i.v.-ENOX nebulisation was evaluated interms of delivered dose, aerodynamic droplet size distribution and in in vitro cell cultures. i.v.-ENOXpresents a high pH (12), and tendency to form crystals in the nebulisation cup. It was determined, based on three different methods (disposable breathing circuit, twin stage impinger and next generation impactor (NGI)) that only 30% of the loaded dose is delivered, and therefore the in vivo effect may be attributed to this low percentage. In vitro deposition characterisationusing an NGI indicated the nebulised droplets had an MMAD of 5 µm and GSD of 2, indicating favourable aerodynamic properties for deposition in the bronchi- alveolar region. Biopharmaceutical evaluations showed that ENOX in the i.v.-ENOX formulation can diffuse through artificial mucus after its deposition. In vitro biological activity was studied, and the results showed that i.v.-ENOX can increase intracellular cAMP levels and protect against oxidative stress in the human lung distal epithelial cell line, NCI-H441, through a local direct effect. Furthermore, the delivered drug permeates across the *in vitro* cell monolayer, suggesting good in vivo bioavailability to achieve a vasodilation effect.

The promising clinical data and the biopharmaceutical characterisation indicated that, despite the inefficiency of the nebulisation process, i.v.-Enoximone had a considerable therapeutic effect. Therefore, an Enoximone-based DPI formulation was assessed. Three cyclodextrins, i.e. β-cyclodextrin (βCD) methyl-β-cyclodextrin, (MβCD) and sulfobutyl ether β-cyclodextrin (SBEβCD), were evaluated as functional excipients. Association constants (Ka) between Enoximone and βCD, MβCD and SBEβCD were 992.3 ± 86.5 M^{-1}, 1795.6 ± 410.4 M^{-1}, and 1623.1 ± 464.3 M^{-1}, respectively. Given the high Ka of all cyclodextrins, solutions containing ENOX, CD, and L-leucine (LL) at pH 12 (βEnox-LL, MβEnox-LL, SBEβEnox-LL) were spray dried. The morphology, as observed by scanning electron microscopy, and the size, as determined by a laser diffraction technique, of the particles showed optimal characteristics for pulmonary delivery, with wrinkled surfaces (minimizing surface contact and particle aggregation[2]), and a median geometric diameter (dx50) of 2.1±0.03 µm, 2.4±0.37 µm, 2.1±0.09 µm, respectively, for βEnox-LL, MβEnox-LL, SBEβEnox-LL. Spray dried composites were characterised by powder X-ray diffraction (PXRD), dynamic vapour sorption and modulated differential scanning calorimetry. All powders were PXRD amorphous, and should, therefore, be able to undergo rapid dissolution in the alveolar aqueous mucus layer [3]. The in vitro deposition profile was determined using an NGI. All powders showed excellent characteristics for pulmonary drug delivery with an MMAD ofapproximately 3 µm. Moreover, between 65% and 83%of the loaded dose was delivered beyond the mouthpiece and throat to the stages of the NGI, indicating a significantly improved efficiency of the formulation to deliver the drug. The three composite systems were also tested on distal lung epithelial cells and showed a high IC_{50} (5.90, 7.98 and 10.62 mg/mL, respectively, for SBEβEnox-LL, MβEnox-LL and βEnox-LL). Further biological and preclinical investigation will contribute to highlight the importance of future therapeutical application of inhalable Enoximone.

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POSTER N. 68

INTRANASAL ADMINISTRATION OF DMF-LOADED HYBRID NANOPARTICLES FOR THE TREATMENT OF MULTIPLE SCLEROSIS

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Multiple sclerosis (MS) is a neurodegenerative disease that affects the central nervous system (CNS), characterized by inflammatory demyelination at the axonal level [1]. Currently, the dimethylfumarate(DMF) (Tecfidera®) is licensed both by the FDA and EMA as the first-line oral therapy for relapsing- remitting MS (RRMS) [2]. After oral administration, DMF is rapidly metabolized into monomethylfumarate (MMF) [3]. Gastrointestinal (GI) disorders were reported for 43% of patients and caused treatment discontinuations within the first 3 months. [4]. An alternative strategy could be using the intranasal administration route, characterized by rapid and high drug absorption in the systemic circulation and direct nose-to-brain transport of drugs [5]. Herein, the aim of this study was the development of hybrid nanoparticles as an intranasal delivery system of DMF for the treatment of MS. Nanoparticles were prepared using phosphatidylcholine (LP), cholesterol (Chol), poloxamers (P) with (LH and LHD) or without (L and LD) hyaluronic acid (HA) at different concentrations. HA, an anionic polysaccharide, has attracted significant attention as it is biocompatible and non-immunogenic. It can specifically bind to CD44 and RHAMM receptors, which are overexpressed in many forms of inflammation [6]. Furthermore, HA is used in nasal formulations due to its mucoadhesive properties, which slow down the mucociliary clearance, and its penetration enhancer activity, which increases the mucosal absorption of drugs [7]. A modified nanoprecipitation technique was used to prepare the unloaded (L and LH) and DMF-loaded nanoparticles (LD and LHD). Nanoparticles were characterized in terms of size and zeta potential (ZP), morphology and differential scanning calorimetry (DSC). The physical stability of all formulations was studied by evaluating the diameter trend during the time at 4°C; total amount of DMF in LHD and LD was measured for 30 days to evaluate the chemical stability of DMF in dispersion. *In vitro* permeation experiments on LHD, LD and free-DMF were carried out using hydrophilic and hydrophobic membranes to simulate the hydrophilic layer (mucus) and the lipophilic structures (cell membranes and neurons) that the DMF and/or nanoparticles need to cross after the administration.The dimensional characteristics of nanoparticles were affected by the presence of HA as the mean size statistically increased from 209 nm (LD) to 230 nm (LHD); the polydispersity index (PDI) was about 0.60 for L and LD, increasing up to about 1.15 (LH and LHD). Thus, the PDI in LH and LHD increased with the added HA in the formulation with a high molecular weight, as reported in the literature [6]. Also the ZP was affected by HA addition which became more negative: from -2.60 of L to -25.0 mV of LH; the DMF loading decreased the ZP (from 0.28 of LD to -19.6 mV of LHD). The TEM images proved that nanoparticles were spherical but with irregular surfaces: LH and LHD appeared to be constituted of regular parallel threads. Results of thermal analyses evidenced that DMF was encapsulated in the formulations. The size of L, LD, LH and LHD stored at4°C was monitored for up to 30 days; the mean diameter of all formulations did not change significantly over time, indicating good physical stability of the nanoparticles in dispersion. The total amount of DMF in dispersion was about 63% with respect to the amount of DMF used for the preparation, equal to 2.8 mg/ml. The chemical stability of DMF was assured for 30 days only in LHD, while the total DMF in LD decreased to 0.5 mg/ml after 30 days. *In vitro*, permeation studies indicated that LHD increased the amount of DMF permeated with respect to free-DMF, regardless the kind of membrane used. The results indicate that LHD nanoparticles could be a promising candidate for DMF encapsulation aimed to the intranasal delivery. In particular, the HA added in the formulation leads to nanoparticles able to load DMF, stable from both chemical anf physical point of view and to promote DMF *in vitro* permeation through both hydrophilic and hydrophobic membranes.

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POSTER N. 69

COMPANION DIAGNOSTIC AROUND THE WORLD: A COMPARISON BETWEEN DIFFERENT REGULATORY LANDSCAPES

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In the last years, medical progresses in diagnostics and therapeutics have allowed to highlight new aspects in clinical practice, laying the foundations for the so-called "personalized medicine". Personalized medicine mainly uses patients' molecular profile to individualize the correct therapeutic strategy for theright person at the right time [1] or to identify the predisposition to certain disease or to allow for a timely and targeted prevention. In many cases, the deriving treatment is based on predictive biomarkers which are really a critical portion of the drug development process. Identifying these biomarkers carefully is crucial for prescribing the appropriate therapy, which in turn depends on the accuracy of *in vitro* diagnostic tests (IVDs). When IVDs are used to identify patients suitable for specific treatment with a drug, they are generally referred to as companion diagnostics (CDx). [2]. CDx are a particular class of IVDs coupled with a therapeutic drug and aimed at assessing its applicability to a specific class of patients selecting those individuals who can benefit more from a certain treatment and with a lower risk.

Although there is a lot of ongoing research in different therapeutic areas, CDx are still more or less exclusively related to oncology, nevertheless rheumatology was covered in a comprehensive review article [3].

The main purpose of drug-associated CDx assay development is to have a test that can predict whether a patient is likely to benefit from it or not. For many drugs, therefore, CDx assays will assume a central role as a kind of 'crucial' stratification factor, both during the different phases of clinical development as well as after approval, when the drug is routinely used in the clinic. The assay will thus become a kind of 'gatekeeper' as regards the treatment decision and with this central role in mind, regulatory requirements for CDx must be at the same level as for drugs [3].

CDx development combines the pharmaceutical and medical device quality and regulatory requirements. For these reasons the approach must reflect the complexity associated with scientific, clinical, operational, and commercial decision-making. A well-defined regulatory and commercialization strategy is hence crucial andmust be developed once user needs have been established, intended use has been defined, and a qualitymanagement system has been implemented [4]. Until 2017, in EU there was no official consensus in the definition of CDx but the publication of IVDR 2017/746 has almost aligned the U.S. and EU definitions [5].

Moreover, due to the increased use of CDx for pharmaceutical marketing, several countries have developed stricter regulatory requirements. These countries include Canada, Australia, Japan and China [6]. Although regulatory authorities differ slightly in their CDx evaluation, they all have to face a common problem: the absence of a reimbursement system. Currently, reimbursement procedures are different from country to country, even within EU member states. This means that CDx funded in the US may not be reimbursed in the EU and vice versa, and this may be an obstacle to the development of new CDx. [5].

The aim of the present work is to provide a comparison between the regulatory landscapes of the main CDx markets trying to highlight their differences and similarities.

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POSTER N. 70

OLIVE LEAF EXTRACT FOR WOUND HEALING TREATMENT

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Wound Healing Society described a wound as the result of "disruption of normal anatomic structure and function". Recently, different strategies have been developed in order to obtain a faster and less painful wound healing process. The olive leaf extract (OLE) has been extensively studied for its antimicrobial and antioxidant features. Such properties are commonly exploited in wound healing application to resolve inflammation and preserve from infection. For these reason OLE was loaded in microparticles based on (MP) of hyaluronic acid (HA) and in MP of chitosan (CS) to obtain a spray patch for the treatment of wounds in anatomical areas difficult to protect with traditional patches. Microparticles of hyaluronic acid (MpHA-OLE) and chitosan (MpCS-OLE) both loaded with OLE extract were prepared by the spray-drying technique. Polyphenols content determination and degradation assay of OLE was performed. Amorphological analysis was conducted on the microparticles using scanning electron microscopy (SEM). Both microparticles types have a smooth surface and a size of about 5 µm. The cytotoxicity and wound healing tests were performed on fibroblasts (cell line balb/3T3 clone A31). Different particle ratios weretested: MpCS-OLE 100%; MpHA-OLE 100%; MpHA-
OLE 50%: MpCS-OLE 50%; MpHA-OLE 75%: MpCS-OLE 25%; MpHA-OLE 25%: MpCS-OLE 75%. The MpHA50MpCS50 mixture, medicated or not, showed a significant higher wound healing activitythan all the other mixture tested [1]. Furthermore water stress, meaning the lack of water administration, can represent a resource for the production of plants with a higher metabolite content than those grown under normal conditions [2]. For this reason olive leaves extracts of the Giarraffa varieties obtained from trees subjected to water stress (OLE-GS) were used for a following study. The aim of this work was to prepare eye drop formulations medicated with OLE-GS for corneal wound healing. Different chitosan derivatives based on a quaternary ammonium chitosan food grade derivative (QA-Ch 50-190 kDa) conjugated with methyl-βCD (MCD), coded QA-Ch-MCD [3] were applied. The ability of ophthalmic drops based on different prepared polymers medicated or not with OLE-GS to accelerate the healing of corneal wounds was evaluated on a model of corneal cell monolayers ofHCE-T cell line, using the assay proposed for the previously work. A cell viability assay was carried out on the HCE-T cell line, the concentration of 10 µg/ml of QA-Ch-MCD was chosen to produce the polymeric complexes. Mixture between QA-Ch and MCD (QA-Ch/OLE-GS) and Ch and MCD (Ch/MCD) were also prepared based on MCD weight ratio in QA-Ch-MCD. A Scratch test assay was performed in order to select the OLE-GS concentration to use for the development of the polymeric mixture. The concentration of 100µg/ml of OLE-GS was chosen. The samples tested for the wound healing assay were: OLE-GS, QA-Ch- MCD, QA-Ch-MCD/OLE-GS, QA-Ch/MCD, QA- Ch/MCD/OLE-GS, Ch/MCD, Ch/MCD/OLE-GS, Ch,
Ch/OLE-GS, MCD and MCD/OLE-GS. No one of the polymeric solution alone was able to reduce the scratch in a significative way compared to the control. For the polymeric complex, all formulation excepted Ch/OLE-GS were able to improve the scratch closure in a significative way compared to the control. Furthermore, QA-Ch-MCD/OLE-GS was able to accelerate the scratch closure in a better way compared to all other formulation tested and compared to OLE- GS alone. The ability of the tested samples to protect against H2O2 induced oxidative damage was evaluatedon HCE-T cell line. All formulations containing OLE- GS and OLE-GS alone were able to increase cell viability following H2O2 induced oxidative damage. Also the vehicles alone, were able to protect from the oxidative damage except MCD. A Draize test was performed using male New Zealand albino rabbits. TheOLE-GS and QA-Ch-MCD/OLE-GS showed no signs of irritation, swelling or redness after 24 hours. The study of kinetic OLE elimination from tear fluid is ongoing. In conclusion OLE-GS at the tested concentration has been shown to improve corneal wound healing on the model used. Furthermore, when this is used complexed with QA-Ch-MCD there is an even more marked effect. Pretreatment with all tested samples (except MCD) are able to protect against H2O2-induced oxidative damage.

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POSTER N. 71

DESIGN AND DEVELOPMENT OF CLAY-POLYMER MICROPARTICLES FOR THE HEALING OF SKIN CHRONIC WOUNDS

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Skin wound healing is a multiphase process that involves a series of biological responses following an injury. However, if its progression is altered or if an underlying disease (e.g. metabolic disorders, infections) is present, the wound could become chronic, posing significant health problems [1]. Three- dimensional structures, such as microfibers and microparticles, could act as scaffold which stimulate skin reparation [2]. Clay minerals are versatile materials that find applications in several fields.Recently, they have also been employed as additives in the development of such scaffolds [3]. Among these, anionic clays like layered double hydroxides (LDH) have already been reported as drug delivery systems or as polymer additives in materials science forbiomedical purposes. LDH are lamellar clays made up of brucite-like layers formed by di-and trivalent cations with an interlayer region containing charge compensating anions and solvation molecules [4]. The combination of their peculiar structure and chemical composition should contribute to support cellular processes [5].

Therefore, the aim of this work was the development of spray-dried microparticles based on sodium alginateand sodium chondroitin sulfate doped with ZnAl LDH to enhance the skin tissue regeneration. Alginate and chondroitin sulfate are two biocompatible and biodegradable polymers and could therefore supportcell adhesion and proliferation. LDH based on Zn^{2+} were selected since Zn^{2+} proved to enhance wound healing and to be effective as antimicrobial.

LDH containing Zn^{2+} and Al^{3+} were synthesized via co-precipitation using ammonia as an alkaline agent. The LDH were then isolated upon centrifugation and dispersed, without further washing, in water under stirring. Sodium alginate and sodium chondroitinsulfate were added to the dispersion in order to obtain afinal concentration of LDH of 5% w/w. The dispersion was spray dried and the microparticles were then crosslinked with $CaCl_2$ following a protocol reported inliterature [6]. The crystalline structure of the LDH was confirmed via XRD and FTIR and their chemical composition was assessed using ICP-OES. The morphology of both the LDH and the microparticles was evaluated via SEM.

The spray drying process led to the formation of smooth microparticles with a spheroidal shape. The presence of LDH slightly altered their morphology, which was characterized by the hexagonal lamellar platelets visible on their surface. The crosslinking step with $CaCl_2$ allowed to obtain water insoluble microparticles without changes in morphology. However, the different steps of the process led to a partial aggregation of the particles and an increase in surface rugosity. In all cases, the mean hydrodynamic diameters of the microparticles, measured via DLS, ranged from 10 to 15 μm and the LDH doping slightly increased the dimensions of the microparticles.

The presence of LDH in the microparticles was confirmed by XRD and FTIR. It was also possible to quantify and confirm the amount of LDH in the structure by means of TGA.

Cell proliferation assays performed on Normal Human Dermal Fibroblasts (NHDF) showed that the scaffolds improved cell growth over 3 and 6 days and the presence of the LDH seemed to increase cell viability.

To conclude, it was possible to manufacture microparticles based on sodium alginate and sodium chondroitin sulfate doped with synthetic LDH, based on Zn^{2+} and Al^{3+}, and to obtain water insoluble matrixes by crosslinking with $CaCl_2$. These scaffolds proved to support cell proliferation over 6 days and are therefore promising for a future use in skin chronic wounds treatment.

Mechanical properties, ions release, and preliminary *in vivo* characterizations will be performed to investigate the systems safety and efficacy. Furthercharacterizations will be performed to clarify the LDH role in polymeric matrix performance and tissueregeneration.

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POSTER N. 72

DESIGN AND DEVELOPMENT OF SCAFFOLDS FOR TISSUE ENGINEERING VIA CENTRIFUGAL SPINNING

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Tissue engineering is a branch of regenerative medicine that focuses on the design and the development of biological substitutes able to repair or replace a damaged tissue [1].
The most promising innovations in this field are the three-dimensional biocompatible scaffolds [2].
One of the most studied methods to develop scaffolds is the electrospinning; however, this technique shows some limits, such as the low fiber rate, high energy demand and the use of organic solvents [3].
In this scenario, centrifugal spinning became a conceivable alternative in fiber production [4]. This method features with high efficiency, low costs and the adaptability to industrial-scale fiber manufacture [5].
Given these premises, the aim of the present work was the design and the development of zein (Z)/ Eudragit® (EU) fibers via centrifugal spinning.
A pre-formulation study was carried out in order to identify the most suitable solvent and the optimum materials concentrations.
EU was spun alone or in a mixture with Z using a centrifugal spinning prototype, equipped with an AC motor and a rotator system combined with a sample holder. Temperature was set at 40°C during the spinning process.
EU and EUZ fibers were subjected to physicochemical characterizations.
The morphological and dimensional analyses were carried out by scanning electron microscopy (Tescan, Mira3XMU, CISRIC, University of Pavia), and they showed smooth fibers with a wide fiber range diameter from 17 up to 34 μm.
The hydration process, in distilled water overnight, did not show a significant reduction of mean fiber diameter. The overall structure was maintained, indicating that fibers preserved their structuralintegrity.
Contact angle (Contact Angle Meter DMe-211 Plus) was evaluated to assess wettability of the scaffolds.The results showed that decreased contact angle values were observed from EU to EUZ, indicating the EUhighest wettability.
Since the external mechanical forces can easily modify and interfere with the healing process [6], an ideal scaffold for wound healing should keep its 3D structure and recover under repeated mechanical stress.Therefore, a low cycling fatigue stress assay was carried out on aligned fibers through a texture analyzer (TAXT Plus, Stable Microsystems, Italy); 10 loading cycles were performed. Tensile stress values from the first to the tenth cycle showed no statistically significant changes, demonstrating the capacity of the scaffolds to maintain their original shapes.
Moreover, cells proliferation and scaffolds cytotoxicity were evaluated on fibers extracts by means of AlamarBlue™ assay. Normal Human Dermal Fibroblasts (NHDF) were kept in contact with EU and EUZ extracts for 24 up to 72 hours. The test demonstrated that EU and EUZ fibers extracts were biocompatible and did not impair cell proliferation up to 72 h. This result was confirmed by CLSM analyses; confocal images showed fibroblasts having fusiform structure and elongated cytoskeletons even after 72h of contact.
Further investigations are ongoing in order to deeply assess the biocompatibility and cellular adhesion on the scaffolds.

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POSTER N. 73

TRANSFEROSOMES AND MONOOLEIN AQUEOUS DISPERSIONS FOR CUTANEOUS APPLICATION OF FERULIC ACID

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Ferulic acid (FA) is a polyphenol derived from several natural sources now used in many skin care products thank to its antioxidant activity [1]. However, its lipophilic nature makes difficult to administrate this molecule through the skin. In the last decades, lipid-based nanosystems (LBN) have attracted significant interest as matrixes for the release of active molecules aimed to improve their bioavailability and to reduce side-effects [2]. In this this study we examined and compared two distinct LBN, namely Transferosomes (TF) and Monoolein Aqueous Dispersions (MAD), as delivery systems for cutaneous application of FA.
The dimensional and morphological characterization of TF and MAD, performed by PCS and Cryo-TEM, demonstrated the influence of the nanosystems composition as well as of FA encapsulation efficiency determined by UV. The diffusion test results obtained by Franz-cells analysis, shows that nanosystems presenting poloxamer 188 in their composition create a multilamellar system able to control the release of the drug. Moreover, FA loaded MAD displayed a lower diffusion rate compared to TF. The patch test analysis demonstrated that both MAD-FA and TF-FA were safe when applied under occlusive condition for 48 hours. Additionally, the effect of FA nanosystems in term of skin tissue morphology and protection againstoxidative damage was evaluated in human skin biopsies exposed to Ozone (O_3). Indeed, O_3 is one of the most recognized oxidative air pollutants able to react with skin macromolecules such as lipids, proteins etc., inducing oxidative stress reactions culminating in the formation of Reactive oxygen species (ROS) and lipid peroxidation products [3,4]. The hematoxylin/eosin staining, and Immunofluorescenceanalysis suggest that TF composed of poloxamer 188 (TP) and MAD formulations loaded with FA might protect against structural skin damage and the development of an oxidative environment (4-hyroxinonenal (4HNE)) induced by O_3 exposure compared to formulations without the active ingredient.

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POSTER N. 74

MONTMORILLONITE - AND CLINOPTILOLITE -LOADED ELECTROSPUN NANOFIBERS AS ENHANCERS IN FOOD PACKAGING

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Food loss and waste (FLW) is one of the most serious social, economic, and environmental issues undermining our planet sustainability [*1*]. According to Food and Agriculture Organization (FAO), nearly 1.3 billion tons of food are lost along the food supply chain. The increasing demand for new food packaging materials, able to increase food safety and prolong shelf-life could take advantage of nanomaterial science. Inherent permeability of polymeric materials to gases and vapours, and their poor barrier and mechanical properties have boosted interest in developing new strategies to improve these properties [*2*].

Nowadays, the application of clay materials in food packaging is getting attention due to their mechanical, chemical, barrier (against oxygen, carbon dioxide, ultraviolet, moisture, and volatiles) and thermal properties [*3*].

Given these premises, the aim of this work was the design and the development of innovative and sustainable packaging materials to limit the food waste, prolong the shelf-life of food and reduce environmental pollution. At this purpose, PVA-based fibers loadedwith two different natural clays, Montmorillonite and Clinoptilolite, belonging to the family of naturalsmectite and zeolite, respectively, were manufactured by means of electrospinning. Clay minerals were grounded in a mortar, sieved with a 75 µm sieve andthe blends were prepared by the addition of clays to the polymerics blends. To make the fibers insoluble in water, the citric acid was added to the blends as crosslinking agent. The systems were obtained using an electrospinning apparatus (STKIT-40, Linari Engineering, Italy), equipped with a high-voltagepower supply (40 kV), a volumetric pump (Razel R99- E), a 10 mL syringe, and a conductive static collector, covered by aluminum foil. The electrospun fibers were then crosslinked by heating at 150°C for 3 h to activate citric acid.

The systems were characterized using a multidisciplinary approach including morphology, size, and mechanical properties.

The morphology and dimensional analysis were evaluated by means of scanning electron microscopy, the systems were characterized by nanofibers having a smooth surface, with a dimensional range between 400 and 500 nm. Crosslinking by heating did not change the nanofibers' dimensions and preserved the structures upon hydration independently of their composition.

Mechanical properties of the systems were evaluated by means of Texture analyzer (TA-XT plus, Stable Microsystems, Italy) equipped with a 5 kg load cell.

The systems doped with clays showed bettermechanical properties compared to the blank system, due to their multi-layered structure.

Moreover, cytotoxicity test was performed from theISO standard test method (ISO 10993-5:2009, biological evaluation of medical devices) on Caco- 2/TC-7 Human Colon Adenocarcinoma Cell Line in order to assess the biocompatibility of the systems. The biocompatibility was assessed by means ofAlamarBlue™ assay on Caco2 cells, kept in contact with fibers extracts (at different concentrations) up to 24 hours. The results confirm that the systems were biocompatible and non-toxic.

In future studies, these packaging materials will be loaded with antimicrobial compounds able to preserve the quality of food and pH-sensitive compounds (e. g. anthocyanins) which can modulate their color depending on the pH of the solution with which they are in contact.

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POSTER N. 75

INNOVATIVE THERMOGEL LOADED WITH OVOALBUMIN FOR IMMUNOLOGICAL TARGETING OF TUMOR CELLS

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Solid tumors are complex tissues consisting not just of a variety of cancer cells, but also of non-transformed biological components such as fibroblasts, endothelial cells, and immune cells, which form the tumor microenvironment [1]. It was also shown that monocytes and macrophages, like fibroblasts, have the ability to transfer proteins to other cells by MVs mediated transfer, one of which is MHC-I. MHC-I binds peptides from endogenous or exogenous proteins and displays them on the cell surface for recognition by CD8+ lymphocytes [2]. So, taking advantage of this physiological phenomenon, a novel therapeutical approach for solid cancer treatment was investigated. The treatment with activated monocytes loaded with ovalbumin antigen (OVA), allows the transfer of the p-MHC-I complex to the cancer cells, making them target of CD8+ lymphocytes cytotoxic response. Oneof the main issues with this type of immunotherapy treatment is that antigen peptides are rapidly digested, reducing the treatment's efficacy.

The aim of this work is to increase the presence over time of the antigen in the intratumoral site forprolonging the release of the peptide at the site of action. For this purpose, in situ thermosensitive gelling systems was developed, loaded with OVA antigen. The antigen is present in thermogel both in free form and loaded in liposomes, in order to protect them fromdegradation and promoting their internalization through biological mechanisms such as transcytosis and phagocytosis.

The thermogel is composed of Pluronic® F-127 and carboxymethyl chitosan (CMCS) in NaCl solution at 0.4%. Different percentages of the two components was investigated and the best formulation was 14%(w/v) of Pluronic® F-127 and 3% (w/v) of CMCS in 0.4% NaCl. Sol-gel-sol phase transition behaviour of the thermogel was investigated using the tube inverting method at 37°C, and the resulted gelation time was 60 seconds. Furthermore, rheological studies are ongoing.To obtain a sterile gel, 1ml of the formulation was autoclaved and 10 µl of 0.2 µm filtered OVA solution (10 mg/ml) was added. Erosion tests were performedup to 30 hours, comparing the release of free OVA from the gel consisting of only Pluronic® F-127 (A: 20% in 0.4% NaCl) and the chosen formulation (B: 14% Pluronic® F-127 and 3% CMCS in 0.4% NaCl).

The data shows that after 30 hours all the OVA was released from gel A, while from B was released only 61.4% of OVA. OVA is quantified by bicinchoninic acid test (BCA). Preliminary cytotoxicity tests were also carried out on murine fibroblast cell line (BALB/3T3 clone A31) by WST-8 assay, comparing gel A with B. The cell viability studies were performedat 4 and 24 hours. After 4 hours there is no toxicity for formulation B, while for A there is a decrease up to about 50%. Following 24 hours the formulation Breaches a viability of about 65%.

In the meantime, we designed new OVA-loaded liposomes composed by HSPC:DMG-PEG:Chol (3:1:1 w/w). The liposomes were prepared by film rehydration and extrusion sizing. The drug loading wasperformed, obtaining liposomes of average diameter105.3 ± 0.665 nm, PDI 0.071 ± 0.017 and 30% loading rate. Erosion studies and release of OVA loaded into the liposomes from the gel are ongoing. In addition, the release of OVA in the gel, loaded with both free form and loaded liposomes, will be analysed.

In vivo toxicity and efficacy studies on BALB/c mice are ongoing.

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POSTER N. 76

BIOPHARMACEUTICAL ASSESSMENT OF PLASMINOGEN FOR ARDS LOCAL TREATMENT

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Acute respiratory distress syndrome (ARDS) is a serious lung injury complication that is frequently associated with bacterial, fungal, and viral infections, including SARS-CoV-2 viral infections. ARDS is significantly connected with patient death, and its clinical care is extremely difficult, with no effective medication currently available. ARDS is characterized by severe respiratory failure, fibrin deposition in both the airways and the lung parenchyma, and the formation of an obstructive hyaline membrane, which severely limits gas exchange[1]. Plasminogen (PLG) is the inactive precursor of the endogenous plasmin enzyme, and the plasminogen/plasmin system is physiologically responsible for the fragmentation of fibrin clots into soluble and therefore eliminableproducts. In addition to the essential role in fibrinolysis, PLG is involved in various processes, such as the recruitment of inflammatory cells[2].

The first aim of this work is to demonstrate the feasibility of inhaling PLG, proposed as an off-label administration of an eyedrop solution, namely a plasminogen-based orphan medical product (PLG-OMP), and to demonstrate the efficacy of the mesh nebulisation of PLG-OMP in an *in vitro* simulation of clinical administration. Furthermore, the dual role of PLG-OMP, as enzymatic and anti-inflammatory agent was investigated. Secondly, the experience with PLG-OMP gave the basis for studying an inhalable formulation of PLG, through the use of functional and redox stabilising excipients. Firstly, the administration of PLG-OMP was proposed by jet nebulisation but, being a protein, PLG is susceptible to partial inactivation under jet nebulisation[3]. Because mesh nebulisation could provide lower damaging stress but maintain lung-targeted aerodynamic features[4], the assessment of mesh nebulised PLG-OMP wasinvestigated. The nebulisation of the solution was performed using an Aerogen® Solo™ vibrating-mesh nebuliser. Aerosolised PLG displayed an excellent *in vitro* deposition profile, with 90% of the active ingredient impacting to the lower portions of a glass impinger. The nebulised PLG remained monomeric, with no change in glycoform composition and 94% of enzymatic activity maintained. It was observed that PLG-OMP nebulisation with oxygen flow determined an activity decrease. *In vitro* investigations evidenced good penetration of aerosolised PLG through artificial airway mucus, as well as poor permeation across an Air–Liquid Interface (ALI) model of pulmonary epithelium. The results suggest that inhalable PLG has a good safety profile, excluding excessive systemic absorption but with good mucus diffusion. Therefore these data supports the hypothesis of a loco-regional action, limited to the lungs. Most significantly, it was observed that PLG-OMP reversed the effects of LPS activation, on macrophage RAW 264.7 cell line, by reducing the expression of pro-inflammatory cytokines IL-1, IL-6 and TNF-α. Thus indicating PLG's immunomodulatory function in an already established inflammatory state. In addition, the *in vivo* efficacy in ARDS murine model is currently ongoing.

For the inhalable and more stable formulation of PLG, different excipients like Hydroxypropyl-β-cyclodextrin (HPβCD), N-Acetylcysteine (NAC) and Methionine (Met) were tested. Cell viability assays were carried out on human lung adenocarcinoma NCI-H441 epithelial cell line. The IC_{50} found are: 68.5 mg/ml for NAC, 64.8 mg/ml for HPβCD and 3.5 mg/ml for Met. Consequently we prepared formulation with 1 mg/ml of PLG-OMP in presence of the single excipients (5% HPβCD, 1% NAC and 1% Met) and saline solution for iso-osmolarity (3% NaCl). Samples were prepared by medium exchange through filtering membranes (Vivaspin®). PLG aggregation was assessed by light scattering techniques (Zetasizer Nano series Nano-ZS) under protein size distribution algorithm. PLG solutions containing NAC, HPβCD and Met show an estimated molecular weight exceeding 92 KDa, probably due to PLG interaction with excipients, without significant dimerization or fragmentation. The formulations were nebulised in oxygen current and the samples obtained were analysed to verify the maintenance of the PLG activity. The aerodynamic and biopharmaceutical assessment of the investigated formulations are presently ongoing.

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POSTER N. 77

Development of hyaluronic-acid-based redox-responsive hydrogels for delivery of protein-based therapeutic agents in the tumour microenvironment

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Finding innovative methods for delivering protein-based therapeutics is crucial, especially for the treatment of cancer, as most newly produced therapeutic agents, including monoclonal antibodies,have a protein-based structure (1). Due to their great biocompatibility, biodegradability and adaptability, hyaluronic acid (HA) based hydrogels are regarded as suitable delivery platforms for these agents (2). In this project we synthesized thiolated hyaluronic acid (HA- SH) with different degrees of substitution (DS%) and formulated multiple hydrogel platforms for the deliveryof Ig G as a model for immunotherapeutic agents. The thiolated HA polymeric chains are cross-linked by disulphide (S-S) bonds which are redox-labile, making these hydrogels a promising system for the delivery of therapeutics in the reductive tumour microenvironment.

Hyaluronic acid (MW=38 KDa) was thiolated with different DS (30-50-70%) and characterized by HNMR. The modified polymers were used for the preparation of hydrogels. Ig G from sheep serum (MW= 150 KDa) was physically entrapped inside the hydrogels and its release kinetics were studied by Ion exchange HPLC. The redox-responsiveness of the hydrogels was studied by exposure to PBS buffer containing 10mM of l-glutathione reduced. The morphological properties of the hydrogels were studied by scanning electron microscopy (SEM) and the rheological characteristics of the hydrogels were measured by a rheometer instrument (Anton Paar- MCR92).

Results:

The hydrogels of 30-50 and 70 DS% demonstrated different characteristics due to different cross-linking densities. In summary, the hydrogels with 70 DS% had the strongest network structure confirmed by rheology, SEM and degradation studies (Figure1). All the hydrogels demonstrated redox sensitivity with different swelling and degradation behaviour in a normal physiological environment versus the redox environment. Furthermore, high encapsulation efficiency and sustained release of the IgG were demonstrated in the formulations with higher cross-linking.

Conclusions:

Thiolated hyaluronic acid-based hydrogels can provide a stimuli-sensitive platform for the delivery of protein-based drugs in cancer therapy. The thiolation degree of HA plays an important role in various characteristics such as rheological properties, release kinetics and degradation time of the hydrogels. By considering the different parameters such as cross-linking, the concentration of the polymer in hydrogel formulations and the molecular weight of hyaluronic acid, the optimum formulation for the desired release mode of protein-based drugs can be selected.

Figure 1. SEM pictures of the hydrogels with (a) DS 70% and (b) DS 30%

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POSTER N. 78

UPCYCLING SQUALENE AS PENETRATION ENHANCER IN DERMATOLOGICAL AND COSMETIC FORMULATIONS

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Introduction: Squalene is a triterpene, one of the main components of skin surface lipids. It is widely used in cosmetic formulations like antioxidant, moisturizer and emollient. Recovered from shark liver in the past, it is recovered from vegetable sources like olive oil, or microorganisms like *S. cerevisiae*. The aim of the work was the optimization of the extraction method of squalene from white wine lees, rich in S. cerevisiae, the green catalytic hydrogenation in squalane, and the evaluation of both squalene and squalane as penetration enhancers.

Methods: We apply ultrasound-assisted extraction (UAE) to isolate squalene from wine lees and *n*-hexane like a solvent, in an ice bath to avoid the overheating of the sample. The frequency of the sonicator was 20 kHz and the energy input was 97% of the total energy of the sonicator (500 W). A duty cycle with an active interval of 8 seconds was used and 4 different extraction times were tested: 10, 15, 20 and 29 min. The quantification of squalene in the lipidic extracts was carried out with HPLC-DAD [1] . The purification of the squalene was made using a column chromatography, and a green Palladium-Mediated catalytic hydrogenation was made, using 3 different clays: montmorillonite, palygorskite and sepiolite. Samples were characterized by ^{1}H- NMR. Franz cells were used for testing the variability of quercetin permeability in the presence or not of either squalene or squalane. Several squalene-based formulations were then tested.

Results: The quantification of squalene with HPLC-DAD demonstrated that by increasing the extraction time (29 min) it is possible to extract 0.0567 mg/g of squalene. The collected solution after the hydrogenation resulted in colourless oil with a yield of 70% for montmorillonite and 60 and 90% for palygorskite-metal and sepiolite-metal, respectively. The ^{1}H-NMR confirmed the hydrogenation of squalene in squalane, where the signals detected from 0.5 to 2 ppm were related to the structure of squalane. To evaluate the ability of squalene and squalene as penetration enhancers, the membrane permeability *in vitro* test with Franz cells was carried out using as a model molecule, the quercetin with a low skin permeability. The results showed an increase of the permeability of quercetin in the presence of either squalene or squalane.

Conclusions: Wine lees is an ethical source of squalene. With a novel and easy process of extraction and catalytic hydrogenation, it is possible to obtain a high quantity of squalane. Its ability to promote permeation of active compounds through membranes makes the squalane an excellent ingredient for dermatological and cosmetic formulations.

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POSTER N. 79

EXPLOITING THE POTENTIAL OF HYBRID LIPID/POLYMER NANOPARTICLES AS CARRIERS FOR PULMONARY DELIVERY OF RNA THERAPEUTICS

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The ability of nucleic acids (NAs) to specifically modulate gene expression makes them the most straightforward therapeutic approach in the treatment of so-called undruggable disease such as chronic respiratory disease (1). Nonetheless, their therapeutic potential is strongly limited by the need of a delivery system able to assist NAs transport through the extracellular and cellular barriers imposed by the lung. barriers.

Our group has successfully developed hybrid nanoparticles constituted by a polymeric core made of poly (lactic-co-glycolic acid) (PLGA) and modified on the surface with lipids to enhance the biocompatibility of the NPs. Dipalmitoyl phosphatidylcholine (DPPC) was selected since it is one of the major components of the pulmonary surfactant while, to specifically tackle the mucus barrier, 1,2-Distearoyl-sn-glycero-3-phosphoethanolamine-Poly(ethylene glycol) (DSPE-PEG) was chosen (2,3). Hybrid lipid/poly(lactide-co-glycolide) nanoparticles (hNPs) loaded with a siRNA against the NFkB gene were successfully developedand fully characterized for size, PDI and ζ potential. The presence of PEGylation did not support any significant change in the main technological features ofthe NPs, while a significant difference was observed in the encapsulation efficiency when polyethylenimine (PEI) was added to the system. Furthermore, the hNPs exhibited a good release kinetics of the siRNA in simulated lung fluids as well as a very good aerodynamic behaviour. Based on the collected results uptake, cytotoxicity, and silencing activity of siRNA-loaded hNPs have been assessed in different human airway cell culture models, providing a tool to optimise hNP properties for in vivo inhalation. A proof of concept of the *in vivo* silencing activity of selectedhNPs was provided in a murine model. Important knowledge was gained on the effect of PEGylation for crossing mucus barrier. Our study highlights how an in-depth knowledge of the barrier that considers also the exacerbation induced by the disease is of outmost importance to determine whether the PEGylation can have a beneficial effect on the crossing ability of the hNPs (3).

Further studies are undergoing to broader the applicability of the optimized hNPs formulation. To adapt the production method to the encapsulation of NAs with higher molecular weight (i.e. mRNA and circular RNA) adding polycations other than PEI to the system as encapsulation enhancers. To develop a final product that can meet the patient's needs, a comparative study between freeze-drying and spray- drying techniques in the presence of different inert carriers, that is mannitol or trehalose, is ongoing (4).

The development of a long-term stable hNP-based nano-embedded microparticles (hNPs NEM) dry powder can increase the patient's compliance to the therapy through a personalised approach. In fact, when the patient's breathing ability allow it, the hNPs NEM can be used as such upon delivery through DPIs. As alternative, the hNPs NEM can be reconstituted with saline solution and delivered through nebulizers. In both cases, the potential of for pulmonary delivery was fully demonstrated upon in vitro aerosolization test prompting toward their validation in vitro or more complex models.

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POSTER N. 80

MARKETING AUTHORISATIONS FOR UNMET MEDICAL NEEDS: A CRITICAL APPRAISAL OF SPECIAL REGULATORY PATHWAYS IN THE EUROPEAN UNION

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INTRODUCTION

Industrial medicinal products intended to be marketed in the European Union must obtain a preventive Marketing Authorisation (MA), issued after a positive opinion by the Competent Authority (CA) and based on the evaluation of a dossier reporting comprehensive quality, nonclinical and clinical data demonstrating the quality, safety, and efficacy of the medicinal product. However, conventional regulatory pathways to the MA may not be effective in ensuring fast patients' access to therapies for rare or life-threatening diseases, or to respond to emergency situations. For this reason, the European legislator has introduced two regulatory pathways for addressing unmet medical needs: the authorisation under "Exceptional Circumstances" (EXC) and the Conditional Marketing Authorisation (CMA). This work aims to discuss the peculiarity of such regulatory pathways and assess the impact of their application on products' market access and penetration since they were entered in force to 2022.

REGULATORY FRAMEWORK

The EXC, ruled by Regulation (EU) No. 726/2004 [1] and Directive 2001/83/CE [2], provides a route for medicinal products to be authorised when clinical data are not complete, and are not expected to be obtained at the time of MA for objective and verifiable reasons (e.g., rarity of the therapeutic indications).

The CMA, ruled by Regulation (EU) No. 726/2004 and by Commission Regulation No. 507/2006 [3] provides a regulatory path through which, under certain conditions (e.g., unmet medical needs related to the treatment, prevention, or diagnosis of seriously debilitating or life-threatening diseases), medicines can be authorised although full data are not provided at the time of submission of the dossier. The CMA, however, does not exempt the applicant from providing full clinical data in the post-marketing phase. If the applicant does not comply with all obligations established by the European Medicines Agency (EMA), the products have to be withdrawn from the market. Differently from the EXC, the CMA is not meant to remain conditional indefinitely, but it is to be converted into a non-conditional authorisation as soon as comprehensive clinical data are available.

In both cases, it is expected that the benefit-risk balance of the medicinal product is positive at the time of first assessment.

METHODS

A review of the regulatory history of all medicines authorised with EXC and CMA has been performed on publicly-access or Institutional databases (e.g., EMA official website, Public Health - Union Register of medicinal products, Community Register of orphan medicinal products, TEDDY Network, WHO Collaborating Centre for Drug Statistics Methodology). For CMAs, analysis focuses on the period from 2nd April 2006 [Data of coming into effect of the Regulation (EC) No 507/2006] to 30th November 2022; while, for EXCs it focuses on the period from 1st January 2002 to 30th November 2022. Vaccines authorized by CMAs or EXCs were exclude from the analyses.

RESULTS

Seventy-one CMAs and 51 EXCs were granted in the EU in the considered period (Figure 1). Most granted CMAs referred to medicines indicated for treating tumours, while most of EXCs for alimentary tract and metabolism diseases. Of the 71 medicinal products with a CMA, 29 (41%) were authorised for the treatment of seriously debilitating or life-threatening diseases, and 20 (28%) for use in the paediatric population. On the other hand, 33 medicines with an EXC (65%) are indicated for paediatric population.

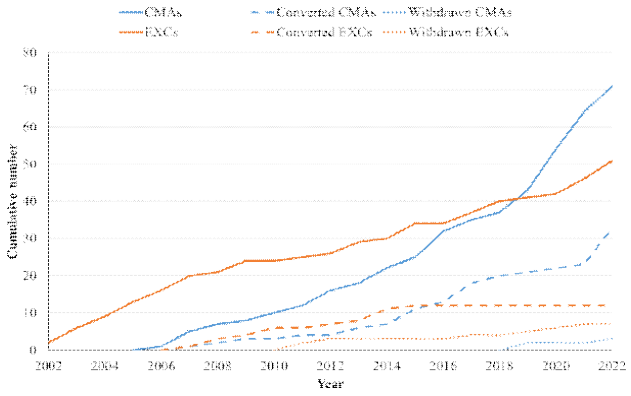

Figure 1. Number of medicines authorized by EMA with CMAs and EXCs (solid lines), converted to normal MAs (dashed lines) or withdrawn (dotted lines).

Focusing on nature of active ingredient, most of CMAs contained small molecules (about 60%), 18 are monoclonal antibodies (mAbs), and one was qualified as Advanced Technology Medicinal Products. A similar trend can be observed for EXCs: about 39% of products

have been manufactured by means of biotechnological processes, whereas 57% of them contains small chemical entities.
Since 2002, 7 EXCs (13.7%), and 3 CMAs products (4.2%) were withdrawn. It is noteworthy that such withdrawal percentages are lower than that of medicines authorized by the EMA following the standard centralized procedure (16.3%) between 2002-2022 [4]. In most cases, the withdrawal was due to commercial reasons, whereas a lack of efficacy was documented only in two cases in post-authorization clinical trials: the product benefit/risk balance was reconsidered after the availability of results from additional post-authorization clinical trials that failed in demonstrating the superiority of the medicine versus standard care. Finally, the marketing authorization of one product was not renewed by the MAH, while the obligations were not fulfilled by the applicant in another one.
After the first authorisation and fulfilling the post-marketing obligations, 33 CMAs switched into "normal" MAs. The average time for switching to a "normal" MA is 4.0 ± 2.7 years. However, the switching period differ significantly among CMAs based on the availability of phase III clinical trials at the moment of CMA granting (Table 1), being lower for products with ongoing phase III trials and higher for products with completed or absent phase III trials.

Phase III trials	CMA applications (n=33)	Conversion time (years)
No	14 (42.4%)	3.3 ± 2.2
Yes, ongoing	5 (15.2%)	2.4 ± 1.6
Yes, complete	14 (42.4%)	5.3 ± 3.1

Table 1. Availability of results of phase III trials at submission of first application of CMA-granted medicines and resulting conversion time to "normal" MA.

DISCUSSION

Our study shows that the CMA and EXC pathways do not pose reasonable concerns for patients' health regarding efficacy and safety with respect to a standard assessment process. Indeed, the withdrawal rate of both CMAs and EXCs do not depart from trends of medicines authorized by the EMA following the standard centralized procedure. Of course, having a complete profile of a medicine is essential, and any CMA is meant to be converted into a "normal" MA as soon as the missing data are available. However, some CMAs have been renewed several times before conversion into "normal" MAs. For some medicines, the CMA have been renewed more than 7 times. Such delays are generally due to challenges in completing clinical trials and/or difficulty in patients' recruitment. In some cases, the trials duration is longer than the 1-year CMA validity.
In this context, other possible strategies should be singled out to speed up the conversion time of CMAs in "normal" MAs. Firstly, available real-world data on efficacy and safety could be used in support of the benefit/risk assessment for CMA products [5]. Secondly, in presence of evident impossibility from the part of CMA holders to provide the missing data, CMA may be converted into EXC. Indeed, for granting an EXC, the MAH should demonstrate to the CHMP that it is impossible to provide comprehensive clinical data for scientific or ethical reasons. In the case of long-standing CMAs such demonstration may be documented by the review of unfulfilled obligations by the MAHs.
Thirdly, these results cast a doubt on the effectiveness of the current CMA pathway, especially in terms of the timing schedule. One-year validity of CMA seems insufficient for fulfilling the regulatory obligations and for providing missing data of clinical trials. If the short-time CMA renewal may be justifiable for the first years to monitor the medicine use, it may result in unjustified costs and overloads for both industrial and regulatory stakeholders in a more mature phase of the CMA. The stretching of CMA validity should be linked to tight monitoring programs in the post-marketing phases to assess trials' progression.

CONCLUSION

CMA and EXC are effective regulatory pathways provided by EU legislator to address unmet medical needs by making available authorised medicines in a brief time, even if comprehensive data are not available. However, a revision of the CMA regulatory pathway seems useful to optimize the conversion rate of CMAs to ensure fulfilment of regulatory obligations for MAHs and avoid unnecessary regulatory costs. As an example, a switch from a fixed one-year validity of the CMA to a flexible time-based scheme (e.g., a one-year validity for the first two years, followed by a two-year validity from the third year) may help striking a balance between the fulfilment of regulatory obligations and products' economic sustainability; otherwise, CMA could be converted into EXC in presence of evident impossibility to provide the missing data.

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POSTER N. 81

CATIONIC HYPER_BRANCHED CYCLODEXTRIN-BASED POLYMERS A NOVEL STRATEGY FOR THE siRNA DELIVERY.

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A promising therapeutic approach that has shown considerable promise in treating both hereditary and acquired disorders is gene therapy. Due to its small size, simplicity in synthesis, potent physiological actions, and availability ofclinical proof from a number of approved siRNA-based medications, interference RNA (iRNA) is one of the most promising novel therapeutic agents in the field of gene materials.
The "naked" RNA must first overcome a number of obstacles before it can start acting [1]. These obstacles include reticuloendothelial clearance, enzymatic degradation, unfavorable biodistribution, cell internalization, and endosomal escape [2].

Over 50 FDA-approved formulations, including cyclodextrins (CDs), have been employed by the pharmaceutical industry for years to get around drug delivery obstacles including solubility, bioavailability, and stability[3]. A macrocyclic ring done up of six, seven, or eight glucose subunits, referred to as -, -, and -cyclodextrin, respectively, compose the CD family of cyclic oligosaccharides [4]. The extensive use of CDs is due to their biocompatibility, capacity for molecular incorporation with host, and ability to purposefully alter the pharmacokinetic profile of drugs.

Our project's goal is to create a novel oligonucleotides delivery system based on Cyclodextrin rather than viral vector from a therapeutic standpoint.
The siRNA used is the SGK1-siRNA (duplex name: mm.Ri.Sgk1.13.2, MW= 16,513 Da, 52 bases)

Our library of -cyclodextrin-based polymers for siRNA delivery application with various hydrophobic moieties was made up of six cationic choline group–grafted alkyl chains. Different polymers with the names BCDI-C0 (non-alkylated), BCDI-C1, BCDI-C5, BCDI-C10, BCDI-C14, and BCDI-C16 that correspond to the increasing number of carbons in the alkyl chain from methyl to hexadecyl had built up by varying alkyl chain lengths.

The results at the pre-formulation study demonstrated a linear relationship between the polymers' alkyl chain length and both their ability to protonate and the efficacy of encapsulating hydrophobic probes. The complexation efficiency, however, was unique to each polymer and did not have a linear connection with the length of the alkyl chain since the interaction between polymers and siRNA involves both electrostatic and hydrophobic mechanisms. In particular,all of the polymers managed to acquire an average particle size of about 200 nm and form polyplex with siRNA. Each polymer reached a plateau in size and Zeta potential at the following N/P ratios: BCDI (N/P=5), BCDI-C1 (N/P=10), BCDI-C5 (N/P=10), BCDI-C10 (N/P=10), BCDI-C14 (N/P=3), and BCDI-C16 (N/P=5). Then, under a variety of conditions, including dilution, physiological temperature, and decomplexation by competing anions, the stability of the polyplexes at N/P = 10 and N/P = 15 was examined.

After 30 minutes of incubation at 37°C, the results showed that all polyplexes retained their size qualities, and alkylated-BCDIs appeared to be a better candidate to stabilize siRNA under dilution. More siRNA was released during decomplexation tests with heparin than with SDS, demonstrating that electrostatic interaction, not hydrophobic interaction, was the primary mechanism for complex formation. At larger N/P ratios, the polyplexes were likewise more stable after competing anion decomplexation. In all stability tests, BCDI-C14 produced the most stable polyplex out of all the BCDIs.

By assessing red blood cell lysis, the hemocompatibility of BCDIs-siRNA polyplexes was assessed. The findings demonstrated that the degree of hemolysis brought on by the polymers or polyplexes at all tested concentrations was comparable to that of the negative control, indicating that the polyplexes are safe for intravenous administration at comparable quantities.
According to the early analysis, all six polyplexes displayed no toxicity on eight tested cell lines at a concentration of 32 nM siRNA and 10 mg/L BCDIs, as indicated by the more than 80% cell viability in every case. The polyplexes were safe even at higher concentrations up to 400 nM SGK1- siRNA (equal to 125 mg/L BCDIs), as deduced from the similarity in GL261 cell viability and proliferation tountreated cells.

This study laids the groundwork for additional research to better understand the function of chemical-physical properties in gene delivery and internalization behavior.

Acknowledgment
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POSTER N. 82

IMPACT OF FREEZE-DRYING ON THE STABIITY OF LIPOSOMES OBTAINED BY ETHANOL INJECTION

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INTRODUCTION

In the production of liposomes, bottom-up approaches present the great advantages of the direct size control and the easiness of implementation. On the other hand, disadvantages arise from the removal of organicsolvents (i.e. ethanol), generally used to solubilize all constituents. When a liposomal formulation has to be dried to improve the long-term stability, lyophilization is the method of choice [1]. Nevertheless, effects of residual solvent, that can influence both the process parameters and products characteristics, have been scantly investigated [2].

This work would contribute to the knowledge of application of freeze-drying to liposomal dispersion containing different contents of residual ethanol by investigating the impact of some auxiliary excipients on the resuspendability of the dried product. The possible interactions with trehalose and poly(vinyl pyrrolidone) K12 (PVP) and the binary mixtures were investigated.

METHODS

Liposome preparation

1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) – cholesterol liposomes sizing around 130 nm (PDI ± 0.17) were prepared by ethanol injection. The residual solvent content was set at 0.1, 1, 3 or 5% as determined by gas chromatography. The compatibility with trehalose, PVP and the binary mixtures (**Table 1**) was assessed at room temperature and after thawing in terms of size, polydispersity index (PDI) and zeta- potential.

Table 1: Size and size distribution of liposome dispersion containing 1% residual ethanol after thawing.

ID	Excipient(s)		After thawing	
	Trehalose:DPPC (molar ratio)	PVP (%)	D_H (nm)	PDI
1	5:1	--0	169±9	0.14±0.06
3	--	0.5	Too dispersed	
4	--	0.75	Too dispersed	
5	5:1	0.5	205±9	0.15±0.01
6	5:1	0.75	Too dispersed	

DSC study on DPPC-excipient interactions

Lipid phase behavior and phase transitions were monitored by DSC (DSC1 Stare System, Mettler Toledo, CH) on DPPC multilamellar vesicles prepared by the thin film hydration method. DPPC films were hydrated with (i) the pure water, afterwards the solution of protectants was added to lamellar phasesamples; or (ii) directly with the solution containing the selected excipient(s).

Freeze-drying

T_g' of protectant(s) in hydro-alcoholic solutions were preliminarily measured by DSC to tailor the process parameters. The samples were freeze-dried using an Epsilon 2–6 LSC plus freeze dryer (Martin Christ, G) according to three different methods (**Table 2**). In all methods, the freezing rate and the heating rate were 1 K/min and 0.1 K/min, respectively.

Table 2. Schematization of process parameters.

Cycle	Freezing	Primary drying	Secondary drying
A	-40 °C 8 h	-40 °C for 48h	25 °C for10h
		0.2 mbar	
B	-48 °C 8 h	-48 °C fro 48h	25 °C for 10h
		0.2 mbar	
C	-48 °C 8 h	-48 °C for 12 h	25 °C for 6h
		-40 °C for 13 h	
		0.1 mbar	

Reconstitution

Freeze-dried products were reconstituted in MilliQ® water and shaken at 100 rpm and 25 °C for 30 min in a benchtop incubation shaker (Sartorius Certomat IS, Varedo, I). Size and zeta -potential were evaluated in samples without visual aggregates.

RESULTS

Compatibility study

All excipients were compatible with liposomes, even if PVP caused the formation of large aggregates after thawing (**Table 1**).

To better understand the protectant effect of DPPC, a DSC study was conducted. DPPC lamellar phase in water presented two endothermic transitions at 34 and 41 °C attributed to the transformation (i) from stable lamellar (Lβ') to hexagonal ripple (Pβ') phase and (ii) from Pβ' to the liquid crystalline (Lα) phase. Trehalose and PVP did not modify DPPC pre-transition significantly (ANOVA ONE WAY, p=0.09). Conversely, trehalose modified the symmetry of the main transition which was slightly skewed on the low temperature side (**Figure 1**). This was concomitant to an increase in the enthalpy of Tm from 55 to 62 J/g suggesting the formation of interactions among f DPPC and trehalose.

After the addition of PVP, the Tm enthalpy massively decreased to 36 J/g, as consequence of the suppression of cohesive interactions between adjacent phospholipid molecules. When DPPC lamellae were hydrated by a solution containing both excipients, the magnitude of enthalpy decrease was lower with respect to PVP and no variation on the onset T_m was found. Consequently, trehalose mitigates the effect of PVP on DPPC bilayer.

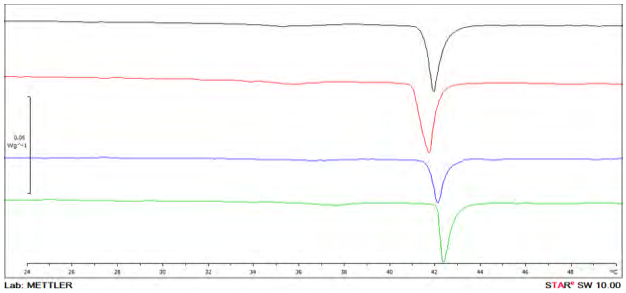

Figure 1 – Thermotropic behavior of DPPC (blackline) in presence of trehalose (red line), or PVP (blue line) or a mixture thereof (green line).

Freeze-dried products

In the co-solvent solution, Tg' of trehalose was depressed and the magnitude of such event was dependent on the residual solvent (**Table 3**).

Table 3. Glass transition of the freeze-concentrated(T_g') solution of trehalose, PVP and the combination thereof, in presence of different ethanol content.

Ethanol (%)	T_g' (°C)		
	Trehalose	PVP	mixture
0	-28.5±1.4	-28.3±0.7	-27.6±0.1
0.1	-29.3±1.5	-32.5±0.5	-30.2±1.1
1	-35.4±1.5	-48.0±3.2	-45.0±0.9
5	-45.5±1.6	_[1]	_[1]

[1] below the limit of detection of the instrument.

In all formulations subjected freeze-drying according to method A (**Table 1**), no macroscopic collapse was observed. However, up to 1% ethanol, the product "blew out" of the vial, probably because of poor cake cohesion upon sublimation.

After rehydration, size and PDI strictly depended on the residual ethanol content and the excipient used and samples containing 1 and 6% residual ethanol presented visual aggregates upon reconstitution. Themodification of parameters in cycle B did not improve the reconstitution.

The degree of destabilization due to freeze-drying stress was lower for formulation freeze-dried according to method C. In fact, the particle size distribution by intensity registered by DLS in reconstituted sample revealed that most of the liposomes maintained the same dimension and lamellarity having before freeze- drying. (DH: 126±6; intensity: 72%). However, the distribution was bimodal with a 18% (in intensity) of aggregates with a particle size of almost 1000 nm. Nevertheless, the last population represented less than one million of the main population of vesicles in number distribution, according to NTA data, which showed that liposome structure was maintained upon freeze-drying even in the presence of 6% v/v of ethanol and a unimodal particle distribution (**Figure 2**).

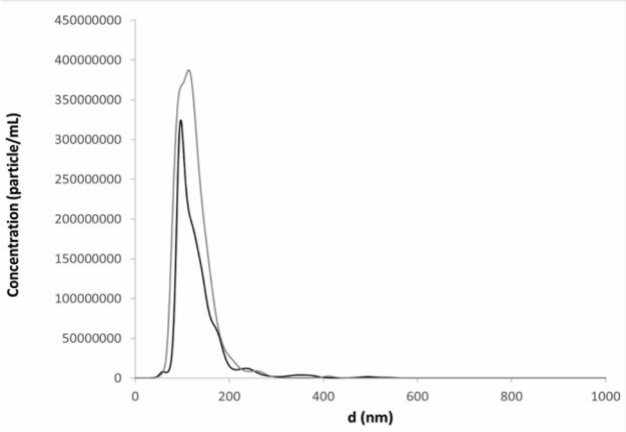

Figure 2- Particle size distribution from NTA measurements of resuspended liposomes in presence of trehalose (black line) and the combination of trehalose and PVP (grey line) after freeze-drying (Method B) a dispersion with 6% v/v ethanol content. This profile was obtained plotted the average data obtained after analyses of 3 reconstituted liposome dispersions.

CONCLUSIONS

A deep knowledge of the formulation and process parameters and the intricated relationship among variables is required to effectively protect liposomes during freeze-drying.

The combination of two excipients, approved for parenteral administration, was effective in preserving liposome structure upon reconstitution of final freeze-dried products with adequate morphology, irrespectively of the ethanol content.

In conclusion, although further studies on a wider range of liposomal formulations are required, this workreports a proof of concept on lyophilization of DPPC liposomes prepared by ethanol injection, avoiding the intermediate step of solvent evaporation and, thus, decreasing the time and the costs of the overall production process of a pharmaceutical form.

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POSTER N. 83

ACTIVATION OF POLYPHENOLIC COMPOUNDS FOR COSMETIC APPLICATIONS USING A GREEN APPROACH

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Food waste is a major waste management and environmental issue in our society. These wastes may have beneficial components like antioxidant molecules that may be recovered and valorized.

This study focuses on fruit peel, an abundant source of antioxidant molecules, to develop a sustainable extraction process that allows for the recovery of these valuable molecules. We developed a novel and eco-friendly method to prepare fermented extracts from fruit peel, exploiting the potential of enzymatic sources to promote the bioconversion of glycosylated molecules into their aglyconic forms [1].

Ultrasonication and enzymatic fermentation were combined to obtain the final extract reached inbioactive molecules. The peel (1 g) was dispersed in 100 mL of solvent and it was sonicated using Ultrasound-Assisted Extraction (UAE) (frequency of20 kHz, amplitude of 95% of the total energy of 500W, 3 min). The extracts were incubated with an enzymatic complex and kept at 37 °C in the incubator for 14 days. All samples were then subjected to sonication, centrifugation, and subsequent analyses at three different time points, G0, G1, and G14, to monitor the samples over time. The evaluation of the formation of the bioactive molecules in the aglyconic form in the fermented extracts was carried out by High-Performance Liquid Chromatography (HPLC-DAD). The results allowed the identification and monitoring over time of the loss of signal related to glycosylated molecules, cyanidin 3-glucoside and pelargonidin 3-glucoside, used as standards. The antioxidant capacity (DPPH, ABTS, FRAP assays), Total Phenol Content (Folin-Ciocalteu assay), cell metabolic activity and cytotoxicity using human fibroblast (HGFs) wereevaluated. We detected an increase in the total phenol content in the samples G0 and G14 which represent the fermented extracts. The antioxidant capacity showed a high antioxidant capacity in G1 and G14 fermented samples. The cell results (Figure 1) revealed that all tested sample doses appeared to be perfectly tolerable by HGF cells, as the cellular vitality rate always exceeds 85%. A statistical reduction in cell toxicitywhen treated with samples of fermented extracts was also detected.

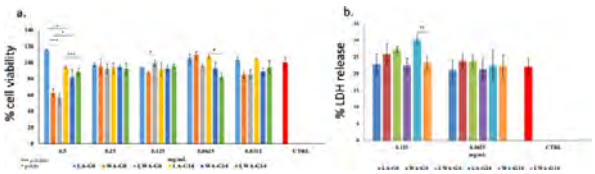

Figure 1. MTT (a) and cytotoxicity (b) assays.

These promising results open up new opportunities in the development of functional products derived from fruit peel, contributing to the sustainable recovery of valuable food resources.

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POSTER N. 84

VISCOELASTIC INJECTABLE HYDROGEL AS DRUG DELIVERY SYSTEM FOR CANCER THERAPY

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Since cancer has been one of the leading causes of death in recent years, research in the development of new treatments always needs progress to improve therapeutic index, bioavailability, andreduction of side effects, as traditional cancer treatments such as chemotherapy, surgery, etc., in the market come with significant side effects due to damage to the surrounding normal tissue [1]. In this study, a biocompatible injectable hydrogel based on the conjugation of hyaluronic acid (HA) and cyclodextrin (CD) was developed as a promising drug delivery system with potential specificity for combined cancer therapy.

Separate synthesis of vinyl sulfone-functionalized CD (CDVS) and thiol-functionalized HA (HASH) were performed as raw materials. The hydrogel was formulated by Michael addition crosslinking between thiol and vinyl sulfone groups at 37 °C [2]. We performed Fourier transform-infrared spectroscopy (FTIR) and RAMAN spectroscopy to understand the chemical composition and crosslinking of the products. Michael addition crosslink and the chemical stability of the gel under reductive environment were demonstrated by a stability control test on gels of HASH with and without CDVS. Scanning Electron Microscopy (SEM) studies revealed the gel morphology and rheological analysis were performedto understand the mechanical behavior of the gel. The encapsulation efficiency of the system was evaluated using both hydrophobic (docetaxel, DTX) andhydrophilic (gemcitabine, GCB) model drugs.

NMR analysis confirms the chemical structure and substitution degree (DS%) of 40% thiol and 30, 55, and 70% vinylsulfone groups of the raw materials. FTIR, RAMAN, and rheology explained the chemical composition and crosslinking behind gelification. SEM revealed a porous morphology. The chemical stability test using dithiothreitol as the reducing agent allowed us to demonstrate that gelification is due to Michel Addiction and not disulphide bond formation, because over time when the gel of HASH with CDVS remained stable, the gel with just HASH got reduced under the reductive environment. Furthermore, preliminary experiments proved that DTX was successfully encapsulated in the CD cavity by host-guest interaction using UV spectroscopy and GCB was trapped during gelification.

These results, furthermore, boosted the interest in analyzing the tailorable physicomechanical properties of the hydrogels in relation to molecular weights (MWs) and (DS%). Different batches of hydrogels were formulated with varying molecular weights of HA (23kDa and 99kDa) and varying DS% of CDVS (30, 55, 70). Developing a highly viscoelastic hydrogel is crucial for its pharmaceutical application as it will possess controlled compliance to restrict cell adherence and mechanical irritation toward local tissues [3]. Rheological results elaborately demonstrated the proportionality and tunable property of the viscoelastic behavior of the hydrogels with varying crosslink densities. The higher the molecular weight and the DS%, the higher the crosslinking density was. With higher crosslinking density, the storage modulus was higher comparatively, and further, the strain exhibited under force was lesser than in lower crosslinked samples.

The high viscoelastic nature and stability of the formulated hydrogels can be anticipated to be potentially advantageous in biomedical and pharmaceutical applications. The studies demonstrate that HA-CD-based hydrogels can be used as a potential platform for combinatorial cancer therapy and, more generally, as a new-generation biocompatible drug delivery system.

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POSTER N. 85

ELASTIN-LIKE RECOMBINAMERS FOR MULTI-MODAL DRUG DELIVERY SYSTEMS

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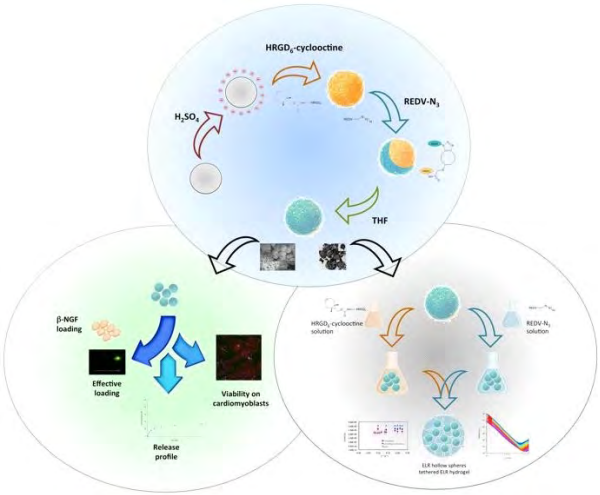
Elastin is a protein with a key role in most of mammalian tissues and it is widely expressed in the extracellular matrix present over myocardium, cartilage and skin. Its elastogenic feature relies on the main cellular components of the tissue network, for instance, endothelial cells, fibroblasts, chondrocytes and keratinocytes [1]. Although elastin is physiologically synthesized at a young age in humans, its lack in natural synthesis causes a disadvantage throughout aging. A clever strategy to overcome such an issue is based on the development of genetically-engineered elastin-mimicking peptides fabrication, so-called elastin-like recombinamers (ELRs). This is an elegant strategy aimed for balancing the low availability ofnatural elastin and fine-tuning the biomaterial structuring and behaviour. Relevant advances in thefield are associated with the investigation of the morphological, mechanical, *in-vitro* and delivery- related properties of ELRs-based systems, fabricated in the form of either hydrogel or microspheres. Different scaffold constructs are studied herein, i.e., microspheres, hydrogel and microsphere-integrated hydrogel, in order to assess their delivery suitability and thoroughly understand the hierarchical complex structuring mechanisms. We used two ELRs (1- $HRGD_6$-cyclooctyne, 2-REDV-N_3) modified with the two different reactive groups needed to form hydrogels *via* a copper-free click-chemistry reaction and functionalized with two different bioactive sequences RGD and REDV that would promote cell adhesion. In this study, the most stable and optimal concentration ratio of ELRs-based hollow spheres exhibited no reduction in cellular metabolic activity. The sacrificial template-based method [2] allowed us to engineer hollow spheres with a first layer of the ELRs $HRGD_6$-component, followed by a second layer of the ELRs REDV-component, by click-chemistry. The ELRs hollow spheres-tethered ELRs hydrogel was prepared by adding the pre-fabricated ELRs hollow spheres priorthe hydrogel click reaction. The hydrogel construct was studied with rheology, NMR and SynchrotronRadiation SAXS (SRSAXS). Hollow spheres were characterized by TEM, SEM, DLS and FT-IR. Drug upload and release were assessed by means of ELISA, confocal microscopy and all constructs were successfully tested for cell metabolic activity, revealing no cytotoxicity.



ELR-based hollow microspheres were fabricated and successfully entrapped into an ELR-hydrogel matrix. Release studies have been conducted to validate the ELRs-based platform suitability as drug delivery system.

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Acknowledgements

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POSTER N. 86

FORMULATION OF CURCUMIN-LOADED NANOMICELLES FOR SKIN MELANOMA TREATMENT

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This study aimed at developing curcumin-loaded nanomicelles for skin application to target melanoma cells, avoiding systemic adverse events.

Curcumin has been well known for centuries for its several pharmacological properties including anti-inflammatory, antimicrobial, anticancer and antioxidant activities [1].

However, curcumin presents several disadvantages; indeed, the molecule is not very stable, due to its chemical structure with reactive groups, and highly insoluble in aqueous medium [2]. To overcome these limitations, we have developed nanomicellar formulations based on binary mixture of surfactantsable to solubilize curcumin in a hydrophilic environment and to extend its stability from autoxidation and photodegradation reactions.

Nanomicelles were obtained by solubilization method, which provided stability and efficient encapsulation of the drug. The choice of surfactants was based on their ability to form stable, safe, and biocompatible nanomicelles. Different types of surfactants were tested for this study, finally selecting Vitamin E-TPGS and Kolliphor ELP.

Vitamin E TPGS is approved as adjuvant in drug delivery systems by FDA and widely reported in literature for topical use. Similarly, Kolliphor ELP has been used safely in dermatological formulations at concentrations up to 4% (w/w)[3],[4].

The formulations were subjected to characterization in terms of pH value, size by dynamic light scattering, amount of curcumin solubilized and encapsulation efficiency by HPLC analytical method. Furthermore, to optimize the nanomicellar formulation development phase, we have settled a suitable Design of Experiment (DOE) study to evaluate the effect of two surfactant's ratios in drug loading, size, and wettability and to select the best performing formulation.

Then, the nanomicelles were analysed by Fourier-transform infrared spectroscopy (FTIR) to evaluate the interaction of curcumin with the surfactants and assess the encapsulation of curcumin inside the lipophilic core of nanomicelles.

Stability studies of the selected formulation were carried out in different conditions in terms of lightexposure and temperature to investigate both the stability of the formulation itself and of the encapsulated curcumin.

Besides, to reach the goal of developing a nanomicellar system that could efficiently deliver curcumin to the skin, *in vitro* release studies and *in vitro* cutaneous permeation studies were performed both atphysiological pH and acidic pH to simulate tumoral environment.

Finally, the cytotoxicity of curcumin-containing nanomicelles was evaluated on melanoma cell lines to investigate the activity of the formulation against skin cancer.

The results of this work will be presented in the poster.

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POSTER N. 87

DOXORUBICIN LOADED LIPOSOMES IN HYDROGELS: RHEOLOGICAL PROPERTIES AND DRUG RELEASE PROFILES

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Hydrogels are versatile formulations that have gained over years a large attention for a wide range of applications in different health-related fields such as tissue engineering, regenerative medicine and drug delivery [1]. In drug delivery, hydrogels were widely investigated as aqueous formulations able to control the release of the drugs and active substances, entrapped inside their 3D network [2]. Diffusion, erosion or swelling-controlled mechanisms are well-known for several polymeric and fibrillary cross-linked networks, and many works have focused on how the hydrogel cross-linking is able to govern the release profile [3]. However, scarce information is available for therelease of drugs loaded inside liposomes, which are incorporated inside a hydrogel. The so called "liposomes-in-hydrogel" formulations have been developed to sustain drug release longer at the administration site [4,5], but at which extent the liposomes membrane and the cross-linked network can have an impact on the drug release, it has not been fully elucidated. Therefore, the aim of the present work is to investigate the release properties of hydrogel systems incorporating doxorubicin (DOX)-loaded liposomes. For this purpose, different systems were prepared by thickening the commercial liposomal dispersion Doxil® (DOX encapsulation efficiency >95%) with hydroxypropylmethyl cellulose (HPMC) K4M and K100 types at the concentration of 2% w/w and Carbopol® 974 at the concentration of 0.5% w/w. Systems were prepared starting from Doxil® liposomal dispersion to have a final formulation, in which almost all drug (DOX used as a model) is initially encapsulated inside liposomes and it is not present in a free form inside the hydrogel network. Polymers (HPMC K4M, HPMC K100 and Carbopol® 974) were selected according to their different thickening potency and ability to form cross-linked systems in water.

All prepared systems were characterised in terms of rheology by stress and frequency sweep test. Release studies were conducted at 37°C using as medium a 150 mM ammonium chloride buffer at pH 5.5 and 6.5, according to the "dialysis membrane" (using a Type II dissolution apparatus) or the ultracentrifugation method. The release properties of the liposomal dispersions were also investigated as a control. The release amount of DOX was quantified by UV-spectroscopy at 488 nm.

The incorporation of DOX-loaded liposomes into HPMC-based hydrogels did not affect the rheological properties in terms of consistency and viscoelasticity. On the other side, the incorporation of DOX-loaded liposomes into Carbopol ® 974 based hydrogels led to a slight decrease in consistency without affecting the viscoelastic properties of the system. DOX release was strongly dependent from pH. Indeed, drug is release faster from liposomes at pH 6.5 than pH 5.5, reaching the 70% and 30% of drug release, respectively, after 48 hours. This effect was observed both for the liposomal dispersion and all the liposomes-incorporating hydrogels. Moreover, DOX release was also found dependent on the viscosity of the hydrogel. Specifically, less drug was released from the hydrogel having a higher consistency, as those prepared using HPMC K100 and Carbopol® 974 with the respect to those prepared with HPMC K4M at both tested pHvalues. However, the effect of the polymeric matrix in controlling the release is more evident at pH 6.5 than atpH 5.5 for all systems, since at a higher pH the passageof the drug across the liposomal membrane is favourite and a larger amount of drug is released from the core ofthe liposomes inside the matrix. A clear effect exerted by the different polymer was also observed for thehydrogel loaded with DOX as a free drug, used as a reference. By comparing the two release methods investigated (i.e. dialysis membrane and ultracentrifugation) for the liposomes incorporating hydrogels, a higher release was achieved using the ultracentrifugation since the formulation was in direct contact with the release medium, thereby better promoting the polymeric matrix dissolution.

In conclusions, the release profiles of DOX are controlled by both the liposomal membrane and the polymeric matrix, as a function of the differentdiffusion rate of the drug across the phospholipid bilayer and the swelled hydrogel.

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POSTER N. 88

THE ART OF PREPARATIONS OF PFCE ENCAPSULATED PLGA NANOPARTICLES FOR ^{19}F MRI

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Introduction: Prevailing over the toxicity of the currently used gadolinium-based contrast agent (CA), the novel class of fluorine-based perfluorocarbons (PFCE) for fluorine-magnetic resonance imaging (FMRI) is paving the way for new research avenues in molecular and cellular imaging [1]. However, PFC's simultaneous hydrophobic and lipophobic nature obliges the need for a carrier system to form a PFCE-delivery system (PDS) [2]. Having several formulations approved for FDA, polylactic-*co*-glycolic acid (PLGA) polymeric nanoparticles (NPs) are aspirants for PDS. Yet the low interactions of the PFCE with the PLGA can result in random encapsulation of the former and discharge of the PFCE during storage. This prompted us to modify the PLGA with fluorinating end chain that could hold the PFCE better using the ring opening polymerisation technique using novel cerium catalysts. Furthermore, the same catalyst was used to modify PLGA with polyethylene glycol (PEG).

Methods: PFCE was encapsulated in PLGA polymeric NPs (***Fig 1***) under emulsification using sonication, homogenisation, and hybrid technique. Formulations were compared for their sizes, PDI, zeta potential, and the sufficient amount of PFCE was inside the NPs for imaging and relaxation times (T_1 & T_2). Furthermore, PLGA was synthesised by ring-opening polymerisation using the widely used tin catalyst and compared the formulation with a novel eco-friendly, cheap, water-tolerant, and non-toxic alternative represented by Cerium-based salt catalysts. The latter was further used to modify PLGA with PEG (2kDa) and fluorine

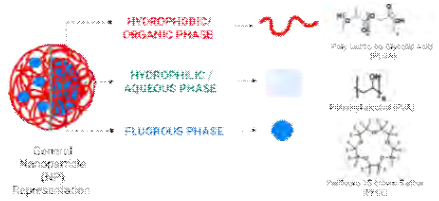

Figure 1: The composition of the nanoparticle - Polylactic-co-glycolic acid (PLGA) as the polymer matrix, Polyvinyl alcohol (PVA) as the surfactant and the Perfluoro-15-crown-ether (PFCE) as the perfluorocarbon

(2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,9 - Heptadeca-1-nonanol). Protein corona studies at a concentration of BSA: NPs at 1:13 in the presence of bovine serum albumin (BSA) was carried out.

Results: Different batches of NP sizes ranging from 170 to 900 nm, PDI from 0.06 to 0.35, negative zeta potential and a loading capacity from 5% to 70% were prepared using the available techniques. Scanning Electron Microscopy (SEM) confirmed the spherical morphology, and SEM associated with Energy Dispersive X-ray Spectroscopy (SEM-EDX) agreed upon the elemental composition of the formulation (***Fig. 2***). Sonication technique gave sizes below 300 nm, low PDI (< 0.25), and relaxation times (T_1 ~ 790 ms and T_2 ~ 270 ms) values specifically for the rapid data acquisition times, and improved signal to noise ratio were promising. Hence, sonication technique was used to study the formulations with PLGA obtained from cerium-based catalysts (PLGA-Ce) that were compared to the commercial ones (made from tin, PLGA-Sn) and also PEGylated PLGA (PLGA-PEG) (***Table 1***). The fluorinated PLGA (PLGA-F) was attempted for the formulation. Protein corona studies carried out up to 24 hours with BSA demonstrated that PEGylated and sonicated PLGA NPs were stable in absolute size value and PDI compared to unloaded NPs.

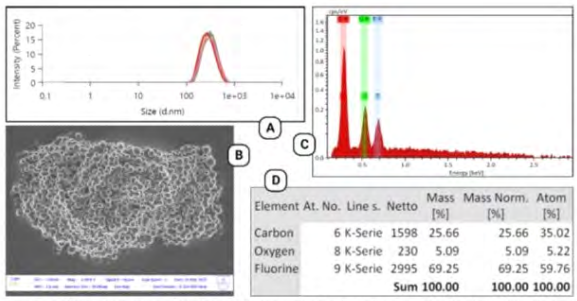

Figure 2: Characterisation of PLGA-PFCE nanoparticles based on **A.** *DLS* **B.** *Scanning Electron Microscope (SEM)* **C.** *Elemental Analysis using SEM-EDX* **D.** *The quantitative EDX analysis spectrum*

Table 1: Various formulations based on their size, PDI, zeta potential and loading capacity. The loading capacity represented shows a decrease in its value

Sl. N	Names	Size (nm)	PDI	Zeta Potential (mV)	Loading Capacity (wt/wt %)
1	PLGA[a]	260 ± 32	0.16 ± 0.0	-21 ± 4	62 ± 21 ? 44 ± 11
2	PLGA-Sn	272 ± 5	0.15 ± 0.0	-24 ± 5	75 ± 15 ? 23 ± 11
3	PLGA-Ce	299 ± 24	0.26 ± 0.0	-26 ± 2	77 ± 8 ? 58 ± 13
4	PLGA-PEG	392 ± 50	0.28 ± 0.0	-26 ± 4	62 ± 9 ? 54 ± 1
5	PLGA-F	548 ± 117	0.69 ± 0.1	-27 ± 4	70 ± 20 ? 56 ± 5

[a] Commercially available from Merck (Mw 7,000 - 17,000 Da, 50:50 the lactide: glycolide ratio)

Conclusion: We have prepared PLGA NPs using various techniques. We also formulated PLGA NPs synthesised using a novel class of cerium catalyst that was further used to tailor PLGA with PEG and fluorine. The feasibility of using the new catalyst for the formulations was demonstrated with NPs smaller than 320 nm, lowerPDI, and the capability to load CA. However, the PLGA-F requires further optimisation. Favourable responses from initial protein corona studies show the possibility to be used *in vivo*, and the relaxation times values validate their suitability as CAs and hence future performance for FMRI.

Acknowledgements: This research is granted by European Commission H2020-MSCA-ITN-2019-NOVA-MRI, H2020-MSCA-ITN-2019-CAST

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POSTER N. 89

Solid dispersions and coground products to valorise oleanolic acid and the pentacyclic triterpenes from Olive leaves.

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Among the secondary metabolites of plants, pentacyclic triterpenes (TTPs) play an important role. The fruits and leaves of *Olea europaea* L. contain a various sterols and triterpenoids. Numerous health benefits have been ascribed to the presence of pentacyclic triterpenes in olive leaves [1]. For this reason, olive leaf bioactive compounds have attracted a growing amount of attention [2].

The leaf contains high amounts of oleanolic acid (3.0-3.5% dry weight), a significant concentration ofmaslinic acid (0.50-0.75% dry weight) and minor levels of ursolic acid (0.20-0.25% dry weight), erythrodiol, and uvaol, (0.05-0.15% dry weight). There is a growing demand of TTPs on the market because they have the potential to be incorporated into newfunctional foods, cosmetics or drugs. However, their poor water solubility, permeability and bioavailability are limiting factors for their therapeutic application.

Oleanolic acid (OA) has various pharmacological activities, such as its antioxidant, antitumor, anti-inflammatory, antidiabetic, and antimicrobial [3]. OA belongs to the Biopharmaceutics Classification System class IV, and it has a very low water solubility (about 1 μg/mL) and poor permeability.

OLEAF4VALUE is a European project aimed to the complete valorisation system for the olive leaves. 4,5 million ton of olive leaves are produced annually in the world by the olive oil industry. This biomass represents a problem for both the farmers and the whole olive oil industry, who need to remove it from the fields and the olive oil mills. Part of this broader European project is the valorisation of this biomass by using the pharmaceutical formulations to improve the biopharmaceutical characteristics of the active compounds of olive leaves. Solid dispersions and coground mixtures were proposed as solutions also considering a possible industrial application.

Solid dispersions (SD) have some advantages over other preparations used in the pharmaceutical technique field. In particular, it is a relatively simple preparation technique that produces the drug in an amorphous form, with a reduced particle size, improved wettability and dispersibility, and high porosity. In this investigation, different polymers, drug-to-polymer weight ratios, and preparation methods were considered [4].

Poloxamer 188, Poloxamer 407, and γ-CD significantly improved the solubility of OA and TTPs, particularly in a 1:2 and 1:5 drug-to-polymer weight ratio, respectively. The solubility increased up 190 μg/mL for OA and 130 μg/mL for TTPs extract. Theoptimized SD were fully characterized using DSC, XRPD, FTIR, and SEM. The results indicated that OA existed in an amorphous form and it was dispersedhomogenously in the polymer. The percentage of dissolution of OA and TTP formulated in gelatine capsules was significantly improved by the binary SD, 2-fold for OA and 5-fold for TTPs.

An increase in the solubility of TTPs and OA was also obtained co-grinding them with different polymers. The solid-state activation was performed in a mill bowl. The mechanochemical activation does not require the use of solvents whose elimination from the activated product can be difficult and expensive. The energy supplied by the mill induced particle size reduction, amorphization and reaction drug-carrier. Na cholate, deoxycholic acid, L-proline, Na CMC, HPMC,mannitol, PEG 4000 and 6000, PVP 40 were the tested carriers using 1:1 and 1:2 active-to-polymer weight ratio. The best results were obtained with Na cholate with 60 minutes of milling time. The aqueous solubilityof OA resulted 670 μg/mL and of TTPs 360 μg/mL. The X-rays and differential scanning calorimetry confirmed the amorphization of OA. The coground products ameliorated the dissolution profile of both OAand TTPs. The improved oral permeability of OA and TTPs extract was confirmed for both the solid formulations using in vitro parallel artificial membrane permeability (PAMPA) assay.

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POSTER N. 90

Thymoquinone-loaded liposomes preparation and their antinociceptive activity in an *in vivo* model of tendinopathy.

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Tendinopathies represent about 45% of musculoskeletal lesions and they are a big burden in clinics characterized by activity-related pain, focal tendon tenderness and intra-tendinous imaging changes. Many approaches have been proposed for tendinopathies' management (e.g., nonsteroidal anti-inflammatory drugs, corticosteroids, eccentricexercises, laser therapy), unfortunately with very little support of efficacy or serious side effects, thus making the identification of new treatments fundamental. The aim of the study was to test the protective and pain reliever effect of thymoquinone-loaded formulations in a rat model of tendinopathy induced by carrageenan intra-tendon injection (20 μL of carrageenan 0.8% on day 1) [1].

Thymoquinone (TQ) is a bioactive constituent of *Nigella sativa* L. with antioxidant and anti-inflammatory activities. Other therapeutic properties are hepatoprotective, cardioprotective, anticancer, antidiabetic, anti-arthritic, neuroprotective and antimicrobial. For these properties, TQ could represent a valid option for tendinopathies' management. Nevertheless, it is hampered by pharmacokinetics characteristics such as short half-life, low biological stability, poor aqueous solubility and low bioavailability. In this study, liposomal formulations were proposed as a biocompatible drug delivery system to improve the solubility and bioactivity of TQ. For the first time, in this study, TQ conventional and hyaluronic acid (HA)-coated liposomes [2] were evaluated in a model of tendinopathy [3]. HA was considered for its physiological role in the homeostasis of tendons [4].

Conventional (LP-TQ) and hyaluronic acid (HA)-coated TQ liposomes (HA-LP-TQ) were characterized and subjected to *in vitro* release and stability studies at 4 °C. The optimized formulation (LP-TQ) contained phosphatidylcholine and cholesterol in a 4:1 weight ratio, and it was able to load 2 and 4 mg/mL of TQ, increasing up to 8-fold its solubility. The optimized systems had good physical and chemical parameters.

Then, the formulations were coated with HA (0.1% w/v), obtaining two preparations with a final TQ concentration of 1 mg/mL (LP-TQ-HA1) and 2 mg/mL (LP-TQ-HA2). The sizes of optimized liposomesranged from 82±1 to 99±5 nm, PdI from 0.15±0.01 to 0.23±0.01, ζ-potential from -20±1 to-44±2 mV, EE% from 65±5 to 73 ±3. All formulations showed a high physical and chemical stability during 5 weeks at +4 °C. Liposomes realized a prolonged release of TQ with respect to the aqueous solution, and in the case of the HA coating, a slower release was obtained compared to the uncoated liposomes. Then, TQ and liposomes were peri-tendon injected (20 μL) on days 1, 3, 5, 7 and 10 to evaluate their antinociceptive profile using mechanical noxious and non-noxious stimuli (paw pressure and von Frey tests), spontaneous pain (incapacitance test) and motor alterations (Rota rod test). HA improved the anti-hypersensitivity effect of TQ with respect to the uncoated formulations and prolonged the effect up to the end of the treatment. Liposomes containing 2 mg/mL of TQ and coveredwith HA (HA-LP-TQ2) reduced the development of spontaneous nociception and hypersensitivity for a long-lasting effect more than the other formulations. Furthermore, HA-LP-TQ2 partially restored the degenerative modifications caused by inflammation as evidenced by the histological findings.

The promising results achieved in this study confirmed the protective and regenerative effects of TQ in addition to its anti-inflammatory properties and its action on collagen production. The liposomes represented a biocompatible formulation indispensable for the delivery of effective doses of TQ through injection. The HA coating also had a positive effect asa pain reliever. In conclusion, the use of TQ encapsulated in HA-LP is suggested as a new injection treatment for tendinopathy management.

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POSTER N. 91

FORMULATION AND CHARACTERIZATION OF LIPID NANOCARRIERS ENCAPSULATING STEROIDAL ALKALOIDS OF TOMATO AND EVALUATION OF POTENTIAL ANTICANCER ACTIVITY IN AN IN VITRO MODEL

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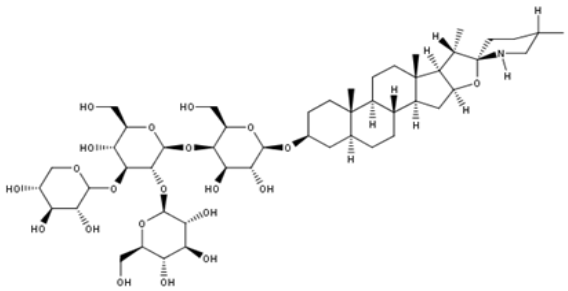
Currently, there is a remarkable attention towards the recovery of by-products of foodprocessing, in particular the plant matrices, as possible sources of bioactive compounds with health properties. It has been shown that the main food wastes contain different bioactive molecules and, for this reason, they can be recovered and re-used in various fields. A typical example is the tomato processing and its by- products. Tomato by-products represent a good source of phytochemical compounds with health properties,such as the steroidal glycoalkaloid α-tomatine (α-TM) and its aglycone tomatidine (TD; Figure 1) [1].

a)



b)

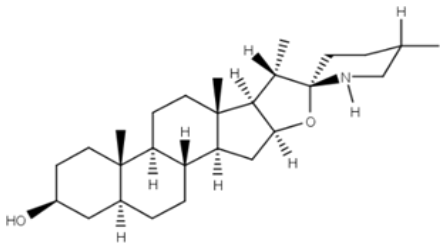


Figure 1. *Chemical structure of (a) tomatine and (b) tomatidine.*

Both molecules have numerous beneficial properties, such as potential anticancer activity [2-5]. Unfortunately, their therapeutic application is limited due to stability and bioavailability issues; thus, reducing their therapeutic effects. Therefore, a valid strategy seems to be their encapsulation into solid lipid nanoparticles (SLN) [6]. SLN are colloidal carriers consisting of solid and biodegradable lipid matrix (Generally Recognized As Safe; GRAS) with an average size between 50 and 1000 nm. The encapsulation of natural products into these nanosystems has been shown numerous advantages, such as protection of the drug from degradation and increased stability and solubility.

Therefore, the aim of this project was to develop innovative nanoformulations containing α-TM (α-TM-SLN) and TD (TD-SLN) for the potential treatment of cancer. Both nanoformulations were prepared by *solvent-diffusion* technique [7] and subsequently characterized in terms of particle size, polydispersity index, zeta potential and calorimetric studies.

The nanotechnological parameters (mean particle size, polydispersity index and zeta potential) were measured by *Dynamic Light Scattering* (DLS) analysis. These data showed that all nanoformulations had an average particle size of about 125 nm, a polydispersity index (PDI) around 0.24 and a zeta potential (ZP) of about -25 mV predicting a good storage stability of the formulations; this data was further confirmed by long-term stability studies (180 days). In fact, these findings showed that all formulations had acceptable long-term stability after 180 days of storage at room temperature. Moreover, unloaded SLN, α-TM-SLN and TD-SLN weresubjected to calorimetric analysis. Unloaded SLN showed a broad, yet well-defined thermogram, with a peak temperature of about 62°C and enthalpy variation of -29 J/g. α-TM-SLN had an enthalpy variation of -25 J/g, a shift of peak at higher temperature (about 65.5°C) and the appearance of a shoulder at 63°C. The results demonstrated that α-TM interacted with SLN, possibly due to its encapsulation in the SLN structure. In particular, the presence of the shoulder suggested a phase separation which could be due to α-TM-rich and poor regions; furthermore, the main peak at higher temperature suggested a stabilization of SLN structure due to the presence of α-TM. Instead, TD-SLNexhibited a unique peak at 64.36 °C with an enthalpy variation of -27.00 J/g which are an evidence of the encapsulation and a uniform distribution of the compound in the SLN.

The release profile of α-TM and TD from the SLN formulations was measured *in vitro* over 24 h

showing that α-TM-SLN and TD-SLN exhibited a slow-release property. This is due to the slow release of molecules which are successfully encapsulated within the lipid core. The results showed that the maximum α-TM (126.4 ng/mL) and TD (31.62 ng/mL) amounts, corresponding to approximately 65% and 88% of the loaded drug, is reached after 22 h followed by a slower release until the end of the experiment, thus indicating a plateau.

Finally, MTT assay was performed to evaluate the cell viability [8] and the potential anticancer activity of both molecules, free and loaded into SLN, using SH-SY5Y cells, a neuroblastoma cancer cellsline, and OECs as normal control cells. MTT results (Figure 2 and 3) indicated that the treatment with free α-TM (0.25 µg/mL) and TD (0.50 µg/mL) induced a significant decrease of the percentage of cell viability when compared with the control. In particular, the effect appeared more evident when SH-SY5Y cells were exposed to the treatment with α-TM-SLN and TD-SLN. Therefore, the use of nanotechnology could be regarded as a promising strategy for delivering α- TM and exploiting its potential anticancer properties.

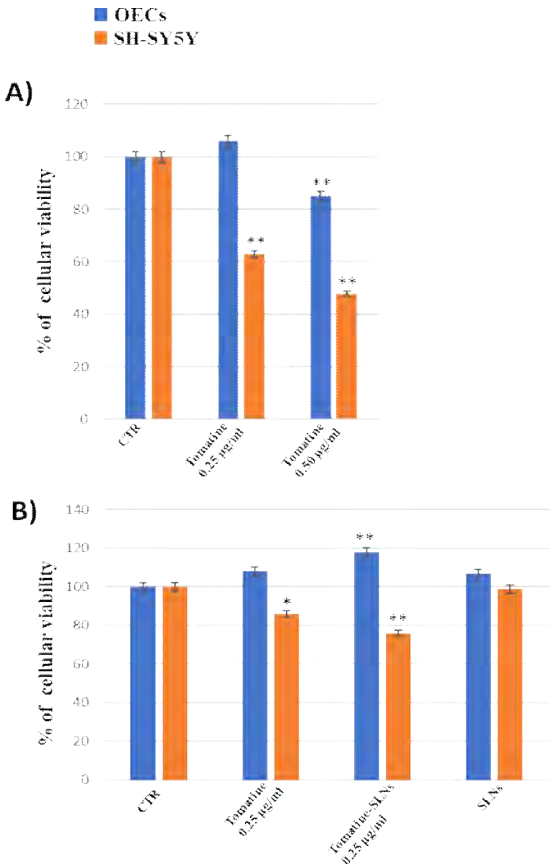


Figure 2. *MTT test performed on OECs (Blue) and SH-SY5Y (orange). (A) Untreated cell (CTR), α-TM at 0.25 µg/mL and 0.5 µg/mL for 24 h. (B) OECs and SH-SY5Y treated with unloaded SLN, α-TM 0.25 µg/mL, α-TM-SLN 0.25 µg/mL for 24 h. * p < 0.05 difference vs. CTR; **p < 0.001 vs. CTR.*

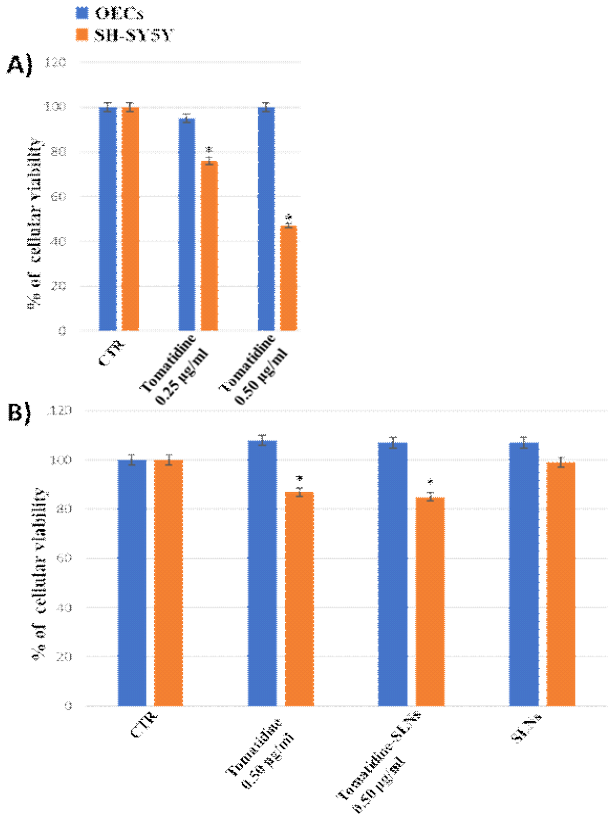


Figure 3. *MTT test performed on OECs (Blue) and SH-SY5Y (orange). (A) Untreated cell (CTR), TD at 0.25 µg/mL and 0.50 µg/mL for 24 h. (B) OECs and SH-SY5Y treated with unloaded SLN, TD 0.50 µg/mL, TD-SLN 0.50 µg/mL for 24 h. * p < 0.05 difference vs. CTR.*

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POSTER N. 92

OPTIMIZATION OF EXTRACTION METHODS, PHYSICOCHEMICAL CHARACTERIZATION AND PRE-FORMULATION STUDIES OF SERICIN OF DIFFERENT MOLECULAR WEIGHTS FOR COSMETIC AND DERMATOLOGIC FORMULATIONS

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Introduction: Sericin is a protein from silkworm cocoons with a molecular weight (MW) ranging from 10 to 400kDa, depending on the extraction method. Sericin of higher MW has been fully characterized, while there is a lack of data concerning the recovery of lower MW fractions and their characterization [1].

Methods: A Central Composite Design was applied to optimize the degumming of sericin from silk waste. Two extraction methods were evaluated, one under heating, and one under sonication. The independent variables were temperature (60-100 °C), extraction time (30-120 min) and solvent (water, urea 8M, Na_2CO_3 0.5% w/w) for the first, amplitude (20-90%), sonication time (3-15 min) and solvent (water, urea 8M, Na_2CO_3 0.5% w/w) for the latter. Among the dependent variables, the yield was calculated, and the MW was determined by sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Sericin of different MW (<30; 30-100; <100kDa) were recovered with Centrifugal Filters (MW cut off: 30kDa and 100kDa). The three fractions were characterized by Scanning Electron Microscopy (SEM), Infrared Spectroscopy (IR), X-ray Crystallography and Dynamic Light Scattering (DLS). Sericin of different MW were formulated as hydrogels by storing 2% w/w sericin solution at 4 °C for different time intervals. The rheological behavior of the fractions was determined by a cone-plate rheometer.

Results: The DoE allowed to select the best method for the recovery of sericin of three fractions of different MW and different properties. The gelation of sericin occurred by the formation of hydrogen bonding between sericin molecules. It has been proved that the network formation is dependent on the temperature and MW [2][3]. Robust gels were recovered when the crosslinking occurred at 4 °C with MW >100kDa sericin. The gelation properties of the formulations were tested and confirmed by rheological measurement.

Conclusions: Sericin with MW <30kDa was used for its capacity to retain water and penetrate up to the stratum corneum. Sericin with MW >30KDa was used to formulate hydrogel.

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POSTER N. 93

FENRETINIDE-LOADED EXTRACELLULAR VESICLES: CHARACTERIZATION AND BIOLOGICAL EVALUATION IN NEUROBLASTOMA 2-D AND 3-D CELL CULTURES

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Neuroblastoma (NB) is the most common pediatric extracranial solid tumour and the outcome for patients with high-risk neuroblastoma remains poor.For this reason, novel treatment strategies are urgently needed.

Fenretinide (4-HPR) is a synthetic retinoid that has shown cytotoxic activity against various solid tumours, meanwhile exhibiting low systemic toxicity and good tolerability in clinical trials in NB patients. However, the main limitation of this molecule relies in its poor solubility and bioavailability, with a consequent limited therapeutic efficacy. As a result, new oral and parenteral formulations are required to improve clinical outcomes. To date several attempts have been made and 4-HPR has been successfully entrapped into liposomes or polymeric micelles [1]. Recently, we opted for an endogenous drug delivery system such as mesenchymal stromal cell-derived extracellular vesicles. Mesenchymal stromal cells (MSCs) have undergone in-depth studies for their therapeutic roles, which appear to be derived from their paracrine activity mostly mediated by extracellular vesicles (EVs). EVs are cell-derived submicronic membranous vesicles that are recognized to be key carriers of information in cell-to-cell communication, exert similar effects as their parental cells and have some of the highly desired attributes of a drug delivery system.

Our aim was to propose a new strategy based on the use of MSC-derived EVs as carrier capable of transporting 4-HPR in NB cells, thus improving its bioavailability.

The study started from MSC isolation from human umbilical cord, then the MSCs were expanded for 15 days and finally exposed to high doses of 4-HPR for different time to assess the best passive drug loading conditions. The resulting 4-HPR-EVs were collected, purified by ultracentrifugation and characterized for size, concentration and loading by Nanoparticle Tracking Analysis and Dynamic Light Scattering. The drug amount encapsulated into the vesicles was determined by HPLC and to estimate the analyte recovery the internal standard N-(4-ethoxyphenyl)-retinamide has been previously synthetized [2].

Loaded and empty EVs showed a concentration of $1.25x10^{11}$ and $1.53x10^{11}$ vesicles/mL and a mean diameter of 120 and 140 nm, respectively. The Zeta potential was comprised between -10 e -15 mV, while the drug concentration was of 6 μM.

Biological studies were performed on a panel of human NB cell lines: IMR-32, HTLA-230, SK-N-AS, SH-SY5Y. Particularly, cells were treated with 1.7 μM 4-HPR-EVs for 48 and 72 h and a time dependent cytotoxic effect was observed with a significant inhibition of cell viability.

Following this, the effects of the free and encapsulated form of 4-HPR on cell viability were compared. Therefore, the most resistant (SH-SY5Y), and the most sensitive (IMR-32) NB cell lines were treated with 1.7 μM 4-HPR-EVs or increasing doses of free 4-HPR (1, 2.5 and 5 μM). The difference was markedly evident in the most resistant cells, where the loaded endogenous carrier showed an improved efficacy over time in inhibiting cell viability compared to free 4-HPR.

To further confirm the increased cytotoxic effect exerted by 4-HPR-EVs, we examined the ability to induce apoptosis by Annexin V-FITC Assay using Flow cytometry. 1.7 μM HPR-EVs were statistically more effective in inducing apoptosis than free 4-HPR, even at the highest free drug concentration tested (5 μM) in SH-SY5Y cells.

Furthermore, incisive results were also attained in a 3-D cell culturing model that better mimics the formation of a tumor mass. 4-HPR-EVs inhibited the viability of IMR-32 and SH-SY5Y spheroids, obtained by seeding cells in ultra-low-attachment 96-well plates, in a statistically significant manner, allowing to appreciate the stronger efficacy of the drug through the EV treatment.

These results demonstrate the anti-tumor effect of 4-HPR-loaded-MSC-derived EVs against NB cells, and underline their potential as a novel drug delivery system against NB, opening to deepened pre-clinical investigations. In this study, we demonstrated for the first time the passive loading capacity of MSCs for the production of 4-HPR-containing EVs. The development of this endogenous carrier for the administration of 4-HPR could overcome its poor bioavailability, which up to now has strongly weakened the clinical success of previous trials.

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POSTER N. 94

DEVELOPMENT OF CHONDROITIN SULFATE AND CHITOSAN-BASED NANOGELS LOADED WITH NARINGENIN-β-CYCLODEXTRIN COMPLEX FOR THE TREATMENT OF DIABETIC RETINOPATHY

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Diabetic retinopathy is the most common complication of diabetes, that is characterized by a progressive bilateral damage of retinal blood vessels. Diabetic eye diseases are commonly treated by intravitreous injections of drugs (i.e., anti-angiogenic agents, steroids, antioxidants and NSAID), which show different limitations, including drug poor penetration and ocular bioavailability. Therefore, the development of new drug delivery systems able to overcome these limits, has gained much attention [1]. Given these premises, the present work aims to develop nanogels, made of chondroitin sulfate (CS) and low molecular weight chitosan (lCH), and loaded with naringenin-? -cyclodextrin complex (NAR/? -CD), to be used for the treatment of diabetic retinopathy. The experimental work was divided into three phases. During the first phase, nanogels were prepared via polyelectrolytic complexation method, starting from two stocks solutions based on lCH (Sigma Aldrich, I) and CS (Bioiberica, I), both prepared in acetic acid 0.1 M. In particular, the CS solution was extruded continuously into the lCH one, under vigorous magnetic stirring [2]. Fifteen different formulations were prepared (from N1 to N15) and different experimental variables were considered: the total polymer concentration (1, 0.75, 0.5 mg/ml), the lCH:CS ratio (1.5:1, 3:1, 1:1 v/v) and the pH value (5.5, 4, 3). Nanogels were firstly characterized in terms of particle size (DLS) and Z potential (ELS) throughout Litesizer 500 (Anton Paar s.r.l. GmbH, A). Thereafter, samples were characterized in terms of morphological properties by scanning electron microscope (SEM; Tescan Mira3 XMU, Brno, CR) after freeze-drying process (Heto DRYWINNER) and transmission electron microscope (JEOL JEM-1200 EXIII, TEM) after solvent evaporation. Finally, in vitro biocompatibility and cellular up-take of the most promising nanogel was assessed on Human Umbilical Vein Endothelial Cells (HUVEC). Afterwards, during the second phase, NAR/? -CD was prepared from two stock solutions of NAR (Sigma Aldrich, I) and ? -CD (Giusto Faravelli, I), prepared respectively in MilliQ water and in absolute ethanol. In detail, NAR solution (2.5 mM) was added to the ? -CD (0.625 mM) one, under mild magnetic stirring (1:1 molar ratio). Such mixture was heated at 60°C for 6 hours and then the solvent was evaporated through vacuum rotary evaporator (Laborota 4000-efficient, Heidolph, DE) [3]. The product was rinsed with MilliQ water, filtered, and freeze dried for 48 h. NAR/? -CD was characterized in terms of complexation efficiency % (CE%) to quantify the amount of NAR within NAR/? -CD and solubility studies were performed by spectrophotometric analysis (Lamba 25 UV/VIS Spectrometer, PerkinElmar Instruments, USA). Moreover, NAR/? -CD morphological properties were investigated through SEM images. Finally, during the last phase the freeze-dried NAR/? -CD was added to the CS solution under magnetic stirring. The subsequent steps for the NAR/? -CD-loaded nanogel preparation were the same as previously described. The loaded nanogels were characterized in terms of DLS and ELS measurements; EE% and LC % were calculated after centrifugation of the nanogel dispersion and spectrophotometric analysis of the supernatant. Lastly, loaded nanogel biocompatibility was evaluated on HUVEC cells. DLS and ELS measurements on empty nanogels revealed that both an increase in the total polymer concentration and lCH:CS ratio resulted in an increase of particle size, while a decrease of pH value was responsible for both an increase in nanogel diameter and Z potential. According to these results, the two most promising samples are N3 (0.5 mg/ml; lCH:CS= 1.5:1; pH=5) and N9 (0.5 mg/ml; lCH:CS= 1:1; pH=5), since they are characterized by the lowest nanogels dimension (lower then 300 nm) and positive zeta potential values, features that should allow the nano systems to reach the retina after intravitreal injection and to be internalized into the cells [4]. Furthermore, studies on HUVEC cells allow the choice of N9 as the best nanogel prototype, because of its higher biocompatibility. Regarding NAR/? -CD, results show a promising CE% equal to 71% and an increase in water solubility (305%) as compared to the non-complexed NAR, resulting exploitable for the subsequent encapsulation within the nanogels. Results of DLS on loaded nanogels report no statistically significant differences between empty and loaded N9, while Z potential slightly increases. In addition, EE% and LC % results indicated the effective loading of nanogel and studies on HUVEC cells confirmed their biocompatibility.

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POSTER N. 96

On the use of Bergamot waxes in the design of lipid nanoparticles intended for cutaneous administration

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INTRODUCTION

According to the National Program for Research 2021-2027, the recovery and valorization of waste and end-of-life organic products through low-cost/low-impact methodologies is one of the main areas of investment. In this view, the use of waste produced by the food industry as novel materials in the pharmaceutical and cosmetic field is attractive. In particular, bergamot peels are an economically valuable source to obtain essential oils for room or body fragrances, but they also contain other components with high added value, such as flavonoids, macromolecules (e.g., pectin), and ascorbic acid. Along with these compounds, which are already extracted from bergamot biomasses and used as functional materials in food supplements or as excipients, bergamot peel is also a source of waxes which can be interesting excipients for the design of formulations to be applied on the skin. Therefore, this work aimed to investigate the feasibility of using bergamot peels as a source of waxes suitable for preparing lipid nanocarriers, such as solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs) for cutaneous application. Two model molecules were used for this purpose: a) DEET, a highly effective insect-repellent molecule, known for its high capacity to penetrate the skin; b) ibuprofen (IB), an anti-inflammatory molecule widely used in commercial topical products.

EXPERIMENTALS

Waxes extraction

The extraction of waxes was conducted by using Soxhlet apparatus. The process was kept under control by monitoring the temperature (200 °C), the speed of the magnetic stirrer (300 rpm), the duration of the extraction and the number of extraction cycles. Then, the waxy components were isolated by cold crystallisation with methanol.

Waxes characterization

Differential Scanning Calorimetry (DSC)

The extracted material was evaluated by DSC (STAR-E, Mettler Toledo, Hong Kong) to investigate the presence of solid or liquid components analyzing the endothermic and exothermic picks. The samples were cooled down from 25 °C to -40 °C at a rate of 5 °C/ min and subsequently heated up to 120 °C at a rate of 2 °C/min.

Gas Chromatography with Mass Selection Detector (GC/MS)

The fatty acids in bergamot extract were analyzed by GC/MS. A method of esterification of the free fatty acids in the extract and transesterification of the long-chain esters were applied to obtain methyl esters corresponding to a quality profile of part of the lipid components. This analysis was carried out using an Agilent mod 7890 gas chromatograph (Agilent Technologies) and an Agilent mod 5975 mass-selection detector (Agilent Technologies).

Nanoparticles preparation and characterization

Nanoparticles were prepared by hot-melt homogenisation. The waxy material that was extracted, either alone (SLNs) or in the presence of myristic acid ester (NLCs) in a 1:5 ratio with waxes [1], was melted at 70 °C for 15 min under magnetic stirring at 200 rpm. The drugs (DEET or ibuprofen) were added to the lipid components during melting. The melted material was emulsified with 5 ml of 1% Kolliphor P188 and sonicated with Ultrasonic Processor (UP200St, Hielsher, equipped with the piezoelectric probe type 2) for 30 min (1 min ON and 30 sec OFF). The emulsion was then cooled down in an ice bath for 15 min to promote nanoparticle formation [2].

At the end, each dispersed system was characterized by dynamic light scattering (Zetasizer, Malvern - UK) and nanoparticle tracking analysis (NTA, Nanosight300, Malvern - UK) to determine the size and polydispersity index and by DSC. The colloidal systems were cooled down from 25°C to -20 °C at a rate of 5 °C/ min and later heated up to 50 °C at a rate of 1 °C/min.

In addition, the loaded nanoparticles were purified by centrifugation and the unencapsulated fraction was calculated by HPLC (Agilent HP 1 100 series) to determine the encapsulation efficiency (EE%).

In vitro skin penetration studies

In vitro permeation tests (IVPT) were performed using Franz diffusion cells in occlusive conditions. Skin samples, obtained from the porcine ears, were carefully mounted on the Franz diffusion cell whose receptor compartment was filled with a) ethanol/water 50/50 for DEET; b) PBS (pH 7.4) for IB. Once the cells were assembled, 300 µL of formulation were loaded in each one. The membrane surface temperature was kept at 32±1°C throughout the experiments. At predetermined times (1, 3, 5, 7, and 24 h), 200 µL samples were withdrawn from the receiver compartment and replaced with clean receiver phase. At the end of each set of experiments, the samples were analyzed by HPLC:

a) at 235 nm, using a Luna® 5µm C18(2), 150 mm X 4.6 mm, Phenomenex (USA).
b) at 225 nm, using a HyperClone™ 5µm BDS C18, 150 mm X 4.6 mm, Phenomenex (USA).

RESULTS AND DISCUSSION

The extracted material from bergamot had a waxy appearance. The DSC analyses revealed the presence of three endothermic events at around 5°C, 25°C and 37 °C, suggesting the presence of liquid components.
The GC/MS data obtained after transesterification of the extracted material evidenced that the main components were C14-C18 fatty acids (**Table 1**).

Analyte name	Peaks area bergamot (%)
Myristic acid	1%
Palmitic acid	54%
Stearic acid	16%
Oleic acid	9%
Linoleic acid	13%
α-linoleic acid	7%

Table 1. Distribution of free fatty acids in bergamot extract.

Based on these data, the extracted material was used as an excipient either alone (SLNs) or in combination with myristic acid ester (NLCs). Regardless of the loading of the active ingredient, these nanoparticles had a Dh of about 200 nm, a concentration of about 1--2 x 10^{12} particle/mL, and a ζ-potential of about -20 mV. Moreover, after the preparation, all the systems resulted amorphous since the only detectable event was the melting of water, indicating that the adopted method of preparation avoided the segregation of the different components present in the bergamot waxes.
Several tests were performed with different drug/wax ratios and EE% was calculated, which was around 36% and 56% for DEET (1:1) and IB (1:3), respectively.
The IVPT were performed on drug-loaded lipid nanoparticles (drug-NLC or drug-SLN) and colloidal systems made of the corresponding placebo nanoparticle and free drug at the same concentrations (Co-administered NLC-drug; Co-administered SLN-drug), using drug solutions at the same concentration (0.3%IB or 0.7%DEET) as a control. **Figures 1 and 2** show the skin permeation profiles of DEET and IB for the different formulations. Surprisingly, the lipid nanoparticles prepared by bergamot waxes acted as skin permeation enhancers, independent of the drug.

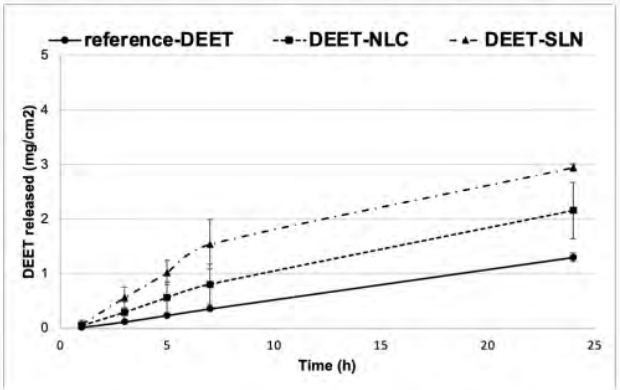

Figure 1. In vitro permeation profiles of formulations loaded with 0.7% w/w DEET.

Indeed, the drug-loaded nanoparticles determined an increase in the flux of at least 8 times and 3 times for IB and DEET, respectively. These data referred not only to NLC, that have been tested for driving the drug through the skin, but also for SLN, that have been developed to retain the drug on the skin surface.

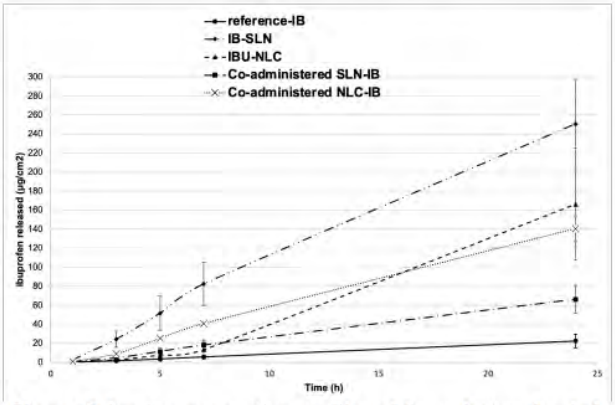

Figure 2. In vitro permeation profiles of formulations loaded with 0.3% w/w ibuprofen.

Thus, the performances of the SLN or NLC alone was tested by adding them to the IB solution, and, once again, the lipid nanoparticles were able to significantly enhance the flux of the drug.

	Enhancer factor
IB-NLC	14.24±5.15
IB-SLN	8.72±3.81
DEET-NLC	2.79±1.31
DEET-SLN	5.32±1.95
Co-administered NLC-IB	6.55±1.81
Co-administered SLN-IB	3.10±0.98

Table 2. Enhancer factor, expressed as the mean flux of each formulation divided by the flux of the control.

CONCLUSION

In conclusion, the preparation of SLN and NLC using bergamot waxes determined an increase in the skin permeation of both DEET and ibuprofen, even though the mechanism of enhancement is yet to be clarified.

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POSTER N. 97

Evaluation of mannose receptor-targeted glycopolymers for the treatment of inflammatory diseases

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INTRODUCTION

The mannose receptor (MR) is a transmembrane glycoprotein expressed by various cells of the immune system, which takes part in the scavenging of endogenous and exogenous molecules and in antigen-presentation. Because of its activities, MR is thought to be involved in the development and exacerbation of different inflammatory and autoimmune diseases, such as rheumatoid arthritis.[1,2] One possible mechanism of MR-mediated inflammation involves the binding and internalization of the enzyme myeloperoxidase (MPO), released by neutrophils recruited in inflamed tissues, which is responsible for the production of reactive oxygen species (ROS) and pro-inflammatory cytokines.[3] The administration of novel sulfo-galactosylated glycopolymers (SG), capable of blocking MR endocytic activity, thus preventing MPO internalization, could in principle reduce the pro-inflammatory effects of the enzyme, having a positive impact on disease progression. Galactosylated glycopolymers (GG), with no binding affinity for MR, were used as negative control.

METHODS

SG and GG were generated by fast Reversible Addition-Fragmentation chain Transfer (RAFT) polymerization and characterized by ^{1}H NMR and GPC.

Bone marrow cells were differentiated into naïve macrophages in presence of M-CSF and then polarized in M_1 and M_2 phenotypes with IFN-γ (50 ng/mL) and IL-4 (10 ng/mL), respectively. For ROS production, M_1 and M_2 macrophages were pre-incubated for 45 min with a 50 µM 2',7'-dichlorofluorescin diacetate solution in HBSS. The solution was then replaced with 600 µM SG or GG solution in HBSS. After 1 h incubation, 25 µg/mL MPO solution in HBBS was added. Fluorescence intensity was measured every 15 min over a 4 h period. Samples incubated with HBSS or MPO only were used as negative and positive controls.

For cytokine detection, both macrophage lines were incubated with a 600 µM SG or GG solution in RPMI+1% of FBS. After 1 h incubation, a 100 µg/mL MPO solution in RPMI+1% of FBS was added to certain wells. Cells were then incubated for 6 h. Supernatants were collected and analyzed for TNF-α and IL-1β detection by ELISA test.

Collagen Induced Arthritis (CIA) was induced in C57BL/6 murine strain following established procedures[4] and treatments started 30 days after the first immunization. SG and GG solution in saline solution or vehicle alone were administered twice a week by intraperitoneal injection. Monitoring of arthritis development was carried out by observational and serological analyses. Blood withdrawals were performed on day 0, 30, 49 and 90 and samples were analyzed for IgG2b and IgG2c quantitation using specific ELISA kits. Histological analyses are ongoing.

RESULTS AND DISCUSSION

SG and GG glycopolymers were obtained via RAFT polymerization (SG, M_n 47.7 kDa, Đ 1.28; GG, M_n 56.3 kDa, Đ 1.35).

Through ROS kinetic assay, we observed a decrease of ROS production of 13.5% and 25% by M_1 and M_2 macrophages, respectively, after pre-incubation with SG followed by addition of MPO, as compared to cells incubated with MPO alone.

Regarding pro-inflammatory cytokines production, IL-1β levels in the samples treated with SG+MPO decreased by 31% in both macrophage phenotypes if compared to the sample stimulated only with MPO, while no difference in TNF-α titers was detected. Moreover, incubation with GG followed by MPO did not show any anti-inflammatory effect, supporting the specificity of the discussed mechanism.

For what concerns *in vivo* testing, no evident worsening of the general health status of mice was detected over 60-day treatment, suggesting the biocompatibility and non-toxicity of SG.

CIA scores, ranging from 1 to 10, with 1 indicating healthy and 10 indicating severely damaged paws, were assigned considering paws thickness, redness, swelling, dactylitis, and ulceration. While in the first 10 days CIA scored increased regardless of the treatment, from day 45 the group of mice treated with SG maintained their mean CIA average score in the range of 6, while the groups treated with saline solution or GG exhibited a further increase, reaching an average score of 8 or higher.

To verify the development and progression of CIA, anti-collagen IgG2 titers were evaluated. After 19 days of treatment with SG, the concentration of IgG2b markedly decreased (< 55%), settling to <49% after further 41 days. IgG2c titers in mice treated with SG dropped by ~27% after 19 days and by ~42% after 60 days of treatment. In contrast, both IgG2 titers almost doubled after 19 days of treatment with GG. Data shows that SG could lower the production of IgG2b-IgG2c involved in the autoimmune progression of joint inflammation.

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POSTER N. 98

ALGINATE-BASED MICROSPHERES CONTAINING ARTEMISIA ABSINTHIUM L. EXTRACT FOR APPLICATION IN BAKED PRODUCTS

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Absinthin, one of the bitter compounds belonging to *Artemisia absinthium* L. (Asteraceae), acts as an agonist of the hTAS2R46 receptor. The latter belongs to TAS2Rs, a family of G protein coupled receptors (GPCRs) involved in the bitter taste perception, expressed in many extraoral tissues, as well as in the mouth [1,2]. Particularly, hTAS2Rs activation in the gastrointestinal tract involves effects that go far beyond the perception of taste as the delay or inhibition of gastric emptying and the modulation of endocrine hormones with the final result of favouring the sense of satiety [3]. The aim of this work was to microencapsulate a dry ethanolic extract of *A. absinthium* into alginate-based beads to obtain a suitable product to insert into biscuits dough, masking the intense bitter taste while preserving the integrity of the compounds to let them target the gastrointestinal bitter receptors exerting their potential activity.

A polymeric solution composed of sodium alginate and Phospholipon® 90G was prepared. Then, an ethanolic dry extract of *A. absinthium,* completely dissolved in ethanol, was added under magnetic stirring to obtain a homogeneous dispersion. Microspheres were formed by dripping the dispersion through two fluid-nozzle into a 100 mM $CaCl_2$ solution, followed by curing, filtration and rinsing. Morphology and size of wet microparticles were investigated by stereomicroscopy and image analysis. Dry microparticles were obtained by fluid bed drying at 25 °C and characterized for morphology by stereomicroscope, SEM and image analysis, particle size distribution by sieving, flowability by the determination of the dynamic angle of repose, residual water by thermogravimetric analysis (TGA). Swelling behaviour in H_2O, HCl pH 1.0 and phosphate buffer pH 6.8 was evaluated and process recovery was calculated. Total polyphenols content (TPC) and antioxidant activity of the absinthe ethanolic extract were determined by Folin-Ciocalteu and ABTS tests, respectively. Bitter compounds, absinthin and anabsinthin, were determined by HPLC-DAD after disaggregation of the microparticles from defatted biscuits and liquid-liquid extraction (LLE).

Wet microparticles were spherical in shape (shape factor SF = 0.890) and showed a smooth surface, while dried ones were still sphere-like (SF = 0.886) but with an irregular surface. The mean diameter of wet microparticles was 2.163 mm and it decreased by more than 50% after drying reaching 0.909 mm. The drying process was successful being the residual water determined by TGA 8.80%. TGA profiles suggest that the presence of absinthe in the microparticles did not affect the thermal degradation profile of their whole structure. The loaded microparticles thermal profile shows the first event at low temperature due to water evaporation, then a remarkable signal between 200-215 °C, attributable to the degradation of alginate and at higher temperatures the degradation of all the other components. There were no signals attributable to absinthe, suggesting that it was loaded into the microparticle structure and protected. ABTS assay revealed an IC50 of 4.80 ± 0.24 mg TE/mL extract; TPC was 11.44 ± 0.34 mg CE/mL extract. The angle of repose value indicated a discrete flowability attitude; the swelling test showed a mild microparticle weight increase without disaggregation in water and HCl for 24 hours and a rapid/significant weight increase with subsequent microparticle disaggregation in phosphate buffer in less than 2 hours. The amounts of absinthin and anabsinthin extracted from the microparticles were respectively 356.8 ± 1.0 ng and 45.0 ± 1.5 ng/g for each gram of dry product.

Dried microparticles were useful as a new ingredient to be included in biscuit dough considering their taste-masking effect. Biscuits containing bitter compounds may be used to decrease the sense of satiety and therefore be applied in some diets for the reduction of caloric intake [4]. Cocoa biscuits, with (type 1) or without (type 2) microparticles, were administered to 11 mean-age voluntary healthy patients and glucose and insulin profile evaluation was carried out at predefined time intervals after the administration. Each patient was double-blindly randomized to consume both biscuit types on two separate days. A Visual Analogue Scale (VAS) questionnaire was completed by the patients before consumption, before lunch and before dinner for the evaluation of hunger, fullness and food desire. The blood glucose level reached after the consumption of the type 1 biscuits was lower than type 2 ones, even though this difference was not statistically significant. VAS results too revealed the absence of significant difference among the variations of hunger, satiety, food desire or satisfaction, even if other ongoing studies suggest type 1 biscuits may be more associated with a reduced hunger and increased fullness in the evening compared to type 2.

ACKNOWLEDGEMENTS

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POSTER N. 99

β-CYCLODEXTRIN NANOSPONGES AS A VERSATILE NANOPLATFORM FOR THE DELIVERY OF ANTI-PANCREATIC TUMOR AGENTS IN COMBINATION WITH HYPHOXIA-BASED ANTITUMORAL STRATEGIES

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Pancreatic ductal adenocarcinoma (PDAC) is the most common malignant tumor of the pancreas with a 5-year overall survival of less than 8%[1]. However, PDAC develops high resistance to conventional chemotherapies and radiation therapy[2].
Indeed, the standard first-line treatment for PDAC, Gemcitabine (GEM), is limited by chemical instability, low bioavailability and chemoresistance. In addition, the clinical use of Olaparib, the PARP inhibitor approved in 2019 as a first-line maintenance treatment of germline BRCA-mutated metastatic PDAC, is limited due to developed resistance mechanisms[3].
Nanotechnology-based drug delivery systems have emerged to play a significant role in increasing the therapeutic efficiency and in overcoming mechanisms of resistance to chemotherapeutic agents[4].
In particular, β-cyclodextrin based nanosponges (NS) are biocompatible nanoparticles obtained by reacting cyclodextrins with suitable cross-linkers, which have been employed to enhance the anticancer activity of both lipophilic and hydrophilic drugs (i.e. doxorubicin, paclitaxel and tamoxifen)[5].
Moreover, NS are able to encapsulate and store oxygen in great extent[6]. Indeed, pancreatic cancer is characterized of a persistent and severe hypoxia, which contribute to the development of therapeutic resistance of pancreatic cancer[7]. To overcome this problem, NS can be used to release oxygen in the hypoxic tumor microenvironment in pancreatic cancer.
The aim of this work was the development of a novel β-cyclodextrin NS formulation as a versatile nanoplatform for the co-delivery of different anti-pancreatic cancer agents (Gemcitabine and Olaparib) and oxygen for pancreatic cancer.
NS nanosuspensions were prepared *via* high pressure homogenization, a top-down method that reduces sizes and forms homogenous NS distribution.
Gemcitabine and Olaparib were loaded in NS and *in vitro* characterized (size and zeta potential determination; morphology evaluation).
Furthermore, ICOS-Fc, a T cell co-stimulatory molecule, was added in GEM-loaded NS triggering an anticancer immune response.
GEM-loaded NS showed a good encapsulation efficiency and a slow and prolonged *in vitro* release kinetics. GEM-loaded NS were tested *in vitro* on pancreatic cancer cell lines. The NS retained the anticancer activity of GEM either on sensitive (CF-PAC, PT45, PANC-02) or resistant (PANC-1 and MIAPaCa-2) pancreatic tumor cells. Furthermore, while GEM promotes cell invasion in PANC-1 and MIAPaCa-2 cells, NS-GEM strongly inhibited it, being the simultaneous treatment with GEM/ICOS-loaded NS even more effective.
In addition, β-cyclodextrin based NS loaded with ICOS might improve the GEM therapeutic efficacy in pancreatic cancer treatment.
Olaparib was successfully loaded in NS forming nanoparticles with a size around 120.5 nm (OLA-loaded NS). *In vitro* biological assays of OLA-loaded NS were performed to evaluate the efficacy of Olaparib on different pancreatic cell lines.
In conclusion, β-cyclodextrin NS saturated with oxygen showed to be an efficient nanocarrier for the delivery of different anti-pancreatic cancer agents in combination with hypoxia-based antitumoral strategies.

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POSTER N. 100

In vitro evaluation of the antioxidant activity of cosmetic emulsions containing pigmented rice polyphenols

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Several topical preparations are formulated with rice (*Oryza sativa* L.) derivatives, like bran, oil, water and flour. Various substances are present in these fractions, such as starch, polyphenols, peptides, silica, phytic acid, vitamins, allantoin, which are highly exploited in the dermatological and cosmetic fields for their proprieties. In fact, their emollient, antioxidant, moisturizing, exfoliating, dermo-repairing activities are widely recognized. In particular, the antioxidant activity is due to the presence of molecules like squalene, γ-oryzanol, vitamin E, carotenoids and polyphenols. Polyphenols are mainly present in rice bran, especially in pigmented varieties, characterized by the presence of colored antioxidant compounds (anthocyanins) [1].

Recently, the set-up of *in vitro* methods for assessing the efficacy of final skin care formulations, not just raw materials, is considered a primary interest.

The present study deals with the formulation of cosmetic emulsions containing rice polyphenols and the *in vitro* evaluation of their antioxidant activity in final products to be used as anti-aging preparations [2].

Various polyphenols extracts were obtained through different procedures and solvents (2-propanol, dimethyl sulfoxide, ethanol) performed on two pigmented rice varieties, the "Artemide" black rice and the "Ermes" red rice, together with the "Apollo" white variety, milled rice where the husk, bran, and germ have been removed. Rice samples were supplied by SA.PI.SE. Coop. Agricola (Vercelli, Italy).

The total phenolic content (TPC) and antiradical activity of each extract were determined according to the Folin-Ciocâlteu method and the DPPH radical scavenging assay, respectively. The extract with the highest TPC and antioxidant activity was selected to prepare O/W emulsions with different emulsifier systems, compositions, and viscosity, by hot or cold emulsifying process. Control emulsions without extract were also prepared.

Emulsions were physico-chemically characterized (appearance, color, pH, viscosity) immediately after their preparation and during a three-month stability test (25 °C, 40 °C; centrifuge, temperature cycles). The assessment of the antioxidant activity of the emulsions was performed by the DPPH$^{\bullet}$ test directly on the prepared emulsions, without prior extraction of polyphenols.

Among the extraction procedures tested, the one performed with aqueous ethanol 50% v/v was selected as the most suitable in terms of performance and dermo-compatibility. As expected, the TPC of white rice extract, was significantly lower than those of pigmented varieties: 10.8 ± 0.9 µg/mL *vs.* 54.4 ± 3.4 µg/mL and *vs.* 243.9 ± 16.9 µg/mL rice extract, expressed as catechin equivalent, for "Apollo", "Ermes" and "Artemide", respectively. A similar trend was observed for the antioxidant activity: 15.4 ± 0.7, 123.6 ± 3.8 and 457.3 ± 24.3 µg Trolox equivalent/mL rice extract. Therefore, the "Artemide" polyphenols extract was used as functional ingredient and added to the emulsions at concentrations from 20 to 40% w/w; the color of the preparations varied from light pink to pale purple (white in the case of the control formulations) and their pH was in the range of 4.70 - 7.10, suitable for topical application. Depending on the emulsifier, the formulations were characterized by different viscosity, with hyper-fluid to thick appearance, not exceeding 6,500 cPs (25 °C, 20 rpm). The most viscous emulsions contained increased percentages of extract, and the surge of viscosity is probably due to the residual presence of fibers and sugars. The emulsions that were stable after three months, and at the same time considered as more performing from a sensorial physical and technological point of view, were subjected to the *in vitro* determination of their antioxidant activity. The results showed that the emulsifying process did not affect the antiradical activity. In addition, this activity was inversely proportional to the viscosity of the final product, ranging from about 24 to 123 µg Trolox equivalent/g emulsion from more viscous to fluid systems. Lower values could be linked to a reduced interaction between polyphenols and DPPH$^{\bullet}$ due to a major thickening and viscosity of emulsions. Therefore, a modified method should be developed for thicker formulations to better evaluate their antioxidant properties and potential use as anti-aging products.

Finally, the use of broken rice (a by-product of rice processing) as a source of natural ingredients for the cosmetic field could be considered a valuable opportunity to enhance local agricultural waste according to the circular economy pillars.

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POSTER N. 101

3D PRINTED COMPOSITE PCL-BASED SCAFFOLDS FOR BONE TISSUE ENGINEERING APPLICATIONS

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Bone tissue engineering scaffolds have emerged as a promising alternative to traditional bone implants, overcoming limitations associated with autografts, allografts, and xenografts. Among various techniques explored for scaffold production, 3D printing (3DP) technologies offer the ability to create objects with complex shapes, geometry, and architecture, providing innovative personalized solutions for a specific patient or a patient group. Among different 3DP techniques, Extrusion-based 3DP techniques, such as Fused Filament Fabrication (FFF), are highly used compared to the others for their highly versatility, feasibility, and cheapness [1]. However, the selection of biomaterials suitable for printing has remained limited. Polycaprolactone (PCL) is a synthetic biodegradable polyester widely investigated for bone scaffold manufacturing due to its biocompatibility, low cost, FDA approval, and ease of processing. Nevertheless, its hydrophobic nature, slow biodegradation, and lack of bioactivity often contribute to implant failures [2]. To address these challenges, the use of structural polymers blended with organic and/or inorganic fillers and bioactive substances has proven to be a simple and effective solution for enhancing performance [3]. Hybrid materials combining the distinct characteristics of individual materials exhibit good processability and unique physicochemical, mechanical, and biological properties.

In this study, we developed PCL-based composite biomaterials through blending with organic and inorganic fillers and drugs. Sodium alginate was selected as a hydrophilic component to improve wettability and biodegradation rate, microcrystalline cellulose was chosen to enhance surface roughness and aid the extrusion process, while nanohydroxyapatite, a bone-like bioceramic, was included to improve osteoconductivity and mechanical properties of the PCL matrix. Additionally, Dexamethasone, an osteoconductive agent, was added to promote bone healing [4].

The composite biomaterials were first produced as films using the solvent casting technique, and then processed into filaments suitable for FFF-3DP via hot melt extrusion. Subsequently, cylindric macroporous scaffolds were 3D printed using FFF. The scaffolds were characterized in terms of physicochemical and technological properties, including size, morphology, surface topography, mechanical properties, swelling ability, degradation profile, and drug release studies. Furthermore, in vitro biological performance was evaluated through hemolysis, cytotoxicity, cell viability, and osteogenic activity assays.

The results demonstrate the effectiveness of blending as an approach to develop novel PCL-based biomaterials for 3D printing of bone scaffolds. All scaffolds exhibited size, 3D architecture, and macroporosity values closely matching the digital model, confirming the good processability of the developed composite biomaterials and the high precision and accuracy of FFF technology. Moreover, the scaffolds maintained the favorable mechanical properties and biocompatibility of PCL (high hemocompatibility and adequate cytocompatibility), while the addition of blending materials successfully modulated critical properties such as wettability, surface roughness, swelling ability, in vitro biodegradation rate, and drug release profiles.

Collectively, this study highlights the combination of PCL as a biomaterial, blending technique, and the utilization of FFF-3DP technology to develop efficient 3D printed bone scaffolds with improved performance.

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POSTER N. 102

MICROPARTICLES OBTAINED BY PRILLING/VIBRATION TECHNIQUE: A SUCCESSFUL STRATEGY TO MEET DIFFERENT PHARMACEUTICAL NEEDS

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A growing challenge in pharmaceutical technology is to develop innovative drug delivery systems that can not only specifically control and target drugs but also improve their administration, overcoming the different problems of patient acceptability and increasing adherence to drug treatment. Multiparticulates, small solid dosage forms with multiple units such as microparticles, have several advantages over traditional single dosage forms (such as tablets and capsules). Microparticles are multi-unit drug delivery systems with well-defined physiological and pharmacokinetic benefits. They ensure greater dispersibility in the gastrointestinal tract, increase bioavailability and reduce the frequency of administration. They represent a flexible and precise dosage form that can be administered safely and completely, and their small size makes them particularly suitable for patients with swallowing difficulties. Among the wide range of production techniques for the preparation of multiparticulates, the most innovative include microencapsulation techniques and, more specifically, the prilling/vibration technique. It works on the principle of laminar jet break up of a liquid stream into equally sized beads/capsules by applying a controlled vibrational frequency to the liquid. The possibility of using a wide range of polymer matrices allows the reproducible and efficient production of monodisperse, homogeneous particles with different drug release profiles.

Presented below are three different case studies, of the research group, in which the use of this strategy proved to be effective and successful in achieving the objectives set.

The first is focused on developing a paediatric formulation as microspheres for colonic budesonide (BUD) delivery for the treatment of inflammatory bowel disease [1]. The two main objectives were to develop a colonic BUD delivery and to explore an oral administration to the paediatric population. To pursue the first objective, we produced a BUD-loaded multiparticulate that can respond to parallel triggered stimuli present in the colon, such as pH, transit time, and resident microbiota. Drug release studies in both simulated gastrointestinal fluid and faecal media showed the response of the microspheres to each of the different triggers confirming the success of our hypothesis. Moreover, the flexibility of dosage, and ease of swallowing of these microspheres having a mean diameter of less than 655 μm, have proposed this formulation for oral administration in paediatric patients.

The second case study aimed to develop dose-adjustable multiparticulates for gastric delivery of misoprostol (MIS) and to increase its stability in this environment. MIS is extremely unstable in the gastric environment and has numerous adverse effects due to its systemic activity [2]. To overcome these problems, MIS was microencapsulated in shell-core microcapsules to protect it and allow gastric delivery. MIS or its complex with HP-β-CD were encapsulated in the core, while the shell was composed of a mixture of selected polymers. The produced formulations showed encapsulation efficiencies higher than 59.86% for microcapsules with MIS only and 97.61% for microcapsules with MIS/HP-β-CD. The diameter of the microcapsules, dose flexibility and ease of repartition, due to good flow properties, make these systems suitable for oral administration in different populations. Release studies conducted in simulated gastric fluids demonstrated the ability of the microcapsules to release and protect MIS into the gastric environment. Indeed, all the formulations provided release MIS within 30 minutes. Furthermore, the presence of the MIS/HP-β-CD inclusion complex stabilized the drug in the acid environment, protecting it from degradation for 2 hours.

Finally, a third case study is underway on microencapsulating probiotics to preserve them during storage and obtain a colon-specific release. Probiotics are particularly sensitive to different conditions such as temperature, humidity, oxygen, gastric acid environment, bile salts, etc. Microcapsules produced ensured high viability and improved stability during storage, protected the probiotics from the gastric acid environment and the presence of bile salts in the intestine, and released them completely and specifically into the colon.

In conclusion, the multiparticulates of the three case studies described demonstrated the potential applications of the prilling/vibration technique. They allowed us to realise different formulation goals, but to achieve that, it was necessary to carry out a careful study of both the process parameters and the selection of excipients and their ratio.

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POSTER N. 103

OLIVE LEAF EXTRACT FOR WOUND HEALING TREATMENT

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Wound Healing Society described a wound as the result of "disruption of normal anatomic structure and function". Recently, different strategies have been developed in order to obtain a faster and less painful wound healing process. The olive leaf extract (OLE) has been extensively studied for its antimicrobial and antioxidant features. Such properties are commonly exploited in wound healing application to resolve inflammation and preserve from infection. For these reason OLE was loaded in microparticles based on (MP) of hyaluronic acid (HA) and in MP of chitosan (CS) to obtain a spray patch for the treatment of wounds in anatomical areas difficult to protect with traditional patches. Microparticles of hyaluronic acid (MpHA-OLE) and chitosan (MpCS-OLE) both loaded with OLE extract were prepared by the spray-drying technique. Polyphenols content determination and degradation assay of OLE was performed. A morphological analysis was conducted on the microparticles using scanning electron microscopy (SEM). Both microparticles types have a smooth surface and a size of about 5 µm. The cytotoxicity and wound healing tests were performed on fibroblasts (cell line balb/3T3 clone A31). Different particle ratios were tested: MpCS-OLE 100%; MpHA-OLE 100%; MpHA-OLE 50%: MpCS-OLE 50%; MpHA-OLE 75%: MpCS-OLE 25%; MpHA-OLE 25%: MpCS-OLE 75%. The MpHA50MpCS50 mixture, medicated or not, showed a significant higher wound healing activity than all the other mixture tested [1]. Furthermore water stress, meaning the lack of water administration, can represent a resource for the production of plants with a higher metabolite content than those grown under normal conditions [2]. For this reason olive leaves extracts of the Giarraffa varieties obtained from trees subjected to water stress (OLE-GS) were used for a following study. The aim of this work was to prepare eye drop formulations medicated with OLE-GS for corneal wound healing. Different chitosan derivatives based on a quaternary ammonium chitosan food grade derivative (QA-Ch 50-190 kDa) conjugated with methyl-βCD (MCD), coded QA-Ch-MCD [3] were applied. The ability of ophthalmic drops based on different prepared polymers medicated or not with OLE-GS to accelerate the healing of corneal wounds was evaluated on a model of corneal cell monolayers of HCE-T cell line, using the assay proposed for the previously work. A cell viability assay was carried out on the HCE-T cell line, the concentration of 10 µg/ml of QA-Ch-MCD was chosen to produce the polymeric complexes. Mixture between QA-Ch and MCD (QA-Ch/OLE-GS) and Ch and MCD (Ch/MCD) were also prepared based on MCD weight ratio in QA-Ch-MCD. A Scratch test assay was performed in order to select the OLE-GS concentration to use for the development of the polymeric mixture. The concentration of 100 µg/ml of OLE-GS was chosen. The samples tested for the wound healing assay were: OLE-GS, QA-Ch-MCD, QA-Ch-MCD/OLE-GS, QA-Ch/MCD, QA-Ch/MCD/OLE-GS, Ch/MCD, Ch/MCD/OLE-GS, Ch, Ch/OLE-GS, MCD and MCD/OLE-GS. No one of the polymeric solution alone was able to reduce the scratch in a significative way compared to the control. For the polymeric complex, all formulation excepted Ch/OLE-GS were able to improve the scratch closure in a significative way compared to the control. Furthermore, QA-Ch-MCD/OLE-GS was able to accelerate the scratch closure in a better way compared to all other formulation tested and compared to OLE-GS alone. The ability of the tested samples to protect against H_2O_2 induced oxidative damage was evaluated on HCE-T cell line. All formulations containing OLE-GS and OLE-GS alone were able to increase cell viability following H_2O_2 induced oxidative damage. Also the vehicles alone, were able to protect from the oxidative damage except MCD. A Draize test was performed using male New Zealand albino rabbits. The OLE-GS and QA-Ch-MCD/OLE-GS showed no signs of irritation, swelling or redness after 24 hours. The study of kinetic OLE elimination from tear fluid is ongoing. In conclusion OLE-GS at the tested concentration has been shown to improve corneal wound healing on the model used. Furthermore, when this is used complexed with QA-Ch-MCD there is an even more marked effect. Pretreatment with all tested samples (except MCD) are able to protect against H_2O_2-induced oxidative damage.

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POSTER N. 104

FORMULATION OF CURCUMIN-LOADED NANOMICELLES FOR SKIN MELANOMA TREATMENT

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This study aimed at developing curcumin-loaded nanomicelles for skin application to target melanoma cells, avoiding systemic adverse events.

Curcumin has been well known for centuries for its several pharmacological properties including anti-inflammatory, antimicrobial, anticancer and antioxidant activities [1].

However, curcumin presents several disadvantages; indeed, the molecule is not very stable, due to its chemical structure with reactive groups, and highly insoluble in aqueous medium [2]. To overcome these limitations, we have developed nanomicellar formulations based on binary mixture of surfactants able to solubilize curcumin in a hydrophilic environment and to extend its stability from autoxidation and photodegradation reactions.

Nanomicelles were obtained by solubilization method, which provided stability and efficient encapsulation of the drug. The choice of surfactants was based on their ability to form stable, safe, and biocompatible nanomicelles. Different types of surfactants were tested for this study, finally selecting Vitamin E-TPGS and Kolliphor ELP.

Vitamin E TPGS is approved as adjuvant in drug delivery systems by FDA and widely reported in literature for topical use. Similarly, Kolliphor ELP has been used safely in dermatological formulations at concentrations up to 4% (w/w) [3],[4].

The formulations were subjected to characterization in terms of pH value, size by dynamic light scattering, amount of curcumin solubilized and encapsulation efficiency by HPLC analytical method. Furthermore, to optimize the nanomicellar formulation development phase, we have settled a suitable Design of Experiment (DOE) study to evaluate the effect of two surfactant's ratios in drug loading, size, and wettability and to select the best performing formulation.

Then, the nanomicelles were analysed by Fourier-transform infrared spectroscopy (FTIR) to evaluate the interaction of curcumin with the surfactants and assess the encapsulation of curcumin inside the lipophilic core of nanomicelles.

Stability studies of the selected formulation were carried out in different conditions in terms of light exposure and temperature to investigate both the stability of the formulation itself and of the encapsulated curcumin.

Besides, to reach the goal of developing a nanomicellar system that could efficiently deliver curcumin to the skin, *in vitro* release studies and *in vitro* cutaneous permeation studies were performed both at physiological pH and acidic pH to simulate tumoral environment.

Finally, the cytotoxicity of curcumin-containing nanomicelles was evaluated on melanoma cell lines to investigate the activity of the formulation against skin cancer.

The results of this work will be presented and discussed in the oral presentation.

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POSTER N. 105

SYNTHESIS AND CHARACTERIZATION OF A NOVEL AMPHIPHILIC POLYACRYLATE DERIVATIVE AS PROMISING MATERIAL FOR PHARMACEUTICAL APPLICATIONS

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Polyacrylic acid (PAA), the synthetic homopolymer of acrylic acid, plays a key role in both pharmaceutical and cosmetic fields, for its rheological and mucoadhesive properties [1]. These features may be enhanced and tuned by means of post-polymerization chemical modifications, leading to new derivatives. In the present work, the preparation, and the characterization of an innovative polymer for potential biomedical, cosmetic, and pharmaceutical applications is reported [2]. Cholesterol (CH) was chosen as the lipophilic moiety to modify the polymer chains due to its low toxicity [3], skin compatibility [4] and the fact that it was already employed in the functionalization of hydrophilic biopolymers such as hyaluronic acid [5].

The derivatization process required several steps: first, PAA was salified with an alkylammonium hydroxide to increase its solubility in an organic environment. CH was then functionalized with a spacer arm, to counteract its steric hindrance [5]. Finally, the reaction of the polymer with the CH derivative yielded the final product "PAAbCH" that consists of CH moieties grafted to a PAA backbone through a spacer group. The degree of derivatization was assessed to be DDr% = 1.8 ± 0.1% through an extensive de-esterification and extraction procedure, followed by HPLC quantification of CH content. The successful reaction and the derivatization degree were separately confirmed by a complete solid-state NMR characterization of both the reactants and the polymer derivative.

The rheological properties of PAAbCH were characterized by recording both steady and oscillatory shear behaviour with a stress-controlled rheometer. PAAbCH showed a strong pseudoplastic behaviour and a zero-shear dynamic viscosity five orders of magnitude higher than non-functionalized sodium polyacrylate (PAANa) at the same 5 mg/ml concentration, 25°C, pH=7. This thickening behaviour may be attributed to the presence of strong inter-chain interactions due to the lipophilic portions grafted onto the backbone of the polymer. The mechanical properties of the samples were evaluated recording the frequency sweep curves: the mechanical spectra suggested a transition from a solution-like to a typical gel-like behaviour from as low as 5 mg/ml. Furthermore, PAAbCH showed promising muco-adhesive properties, investigated by means of a TA-XT2i texture analyser: the test consisted of measuring the force required to detach a polymer disc tablet from a fixed porcine mucin disc tablet. PAAbCH showed also amphiphilic properties: it was able to significantly improve the solubility of poorly water-soluble drug molecules, such as curcumin, piroxicam, and betamethasone valerate. These were dissolved in aqueous PAAbCH solution through a two-step treatment that increased the apparent solubility of the three molecules up to two orders of magnitude, quantified by HPLC. ABTS antioxidant activity assays indicated that solubilized curcumin retained its antioxidant activity, while its availability in solution was improved when compared with the free molecule. Drug release tests were conducted with diclofenac sodium salt, both in solution, against PBS buffer, and transmembrane, employing Strat-M membranes, for intact skin barrier simulation: the release profiles of all the formulations were gradual over the span of 24h, showing also promising signs of permeation enhancement. Finally, o/w emulsion were prepared by deaeration and planetary mixing of 2,5 mg/ml PAAbCH solutions with various V/V sunflower seeds oil fractions: accelerated stability tests indicated an estimated emulsion stability of over one year.

A new amphiphilic derivative of polyacrylic acid, PAAbCH, was synthesized and characterized by solid-state NMR and in terms of its rheological properties. The experimental results indicated that PAAbCH is capable not only of acting as a viscosity agent but can also give physical hydrogels even at low concentrations. Furthermore, PAAbCH significantly increased the water solubility of hydrophobic drug molecules, without changing their chemical properties, and showed promising mucoadhesive and permeation enhancing properties. Thus, PAAbCH can be proposed for both water-based gel preparations and emulsions, to be used in different fields, ranging from pharmaceutics to cosmetics.

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POSTER N. 107

LIPID AND POLYMER-BASED NANOSYSTEMS TO IMPROVE RETINAL DELIVERY OF AXITINIB IN THE TREATMENT OF DIABETIC RETINOPATHY

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Axitinib (AXB) is a multi-receptor tyrosine kinase inhibitor molecule. It acts by blocking the receptors for vascular endothelial growth factor (VEGFR) and platelet-derived growth factor (PDGFR), which are responsible for the development of neovascularization [1-2]. To date, intravitreal injections of anti-VEGF have become the first-line medical treatment for diabetic macular oedema, a degeneration of diabetic retinopathy [3]. Despite the efficacy of intravitreal injections, the complications of this type of administration are manifold [4]. Our study aimed to design a nanotechnological formulation capable of delivering AXB to the retinal tissue after topical administration.

In order to increase AXB bioavailability, two different lipid-based formulations (NLC and SLN) were developed with the use of the experimental design (DoE). I-optimal design was employed to build the experiments. The NLC and SLN were respectively prepared by simple and easily scalable methods: melt emulsification followed by ultrasonication and solvent-injection method.

NLC and SLN were first optimized using response surface methodology (RSM) and then loaded with gradually increasing concentrations of AXB. The highest concentration achieved was 400uM, which was almost 1000 times higher than the water solubility of AXB (0.2 µg/mL).

NLC and SLN were characterized for physico-chemical and technological parameters. A mean particle size below 200 nm, low PDI (<0.300), pH and osmolality values would define the systems as suitable for ocular administration. High EE% was confirmed for AXB-loaded NLC and AXB-loaded SLN by HPLC method.

Another nanosystem was set up with different nature: polymeric micelles (PM), developed according to our previous work [5]. Thin film hydration method was employed for PM preparation. AXB-loaded PM were loaded with the same concentration of AXB 400uM and characterized under the same physico-chemical parameters.

DSC studies were used to confirm the stability between the excipients and the drug for all systems.

Stability studies were performed at different storage conditions up to 6 months according to ICH-Q1A(R2) guidelines. The stability of all formulations was also evaluated by the optical analyser Turbiscan® Ageing Station for 30 days.

AXB-loaded NLC, AXB-loaded SLN and AXB-loaded PM were subjected to pharmacokinetic (PK) studies, to evaluate the ocular pharmacokinetics and tissue distribution of AXB in male New Zealand white rabbits, following a single ocular instillation of AXB test formulations. PK-I study showed better results for AXB-loaded NLC and AXB-loaded SLN. Thus, these nanosystems were used for further studies. In order to increase the efficiency of the formulations, two strategies were set up: the first strategy involved the lyophilization and redispersion of the powder in half volume of aqueous medium than the initial one to double the final concentration of AXB, up to 2mM. This strategy was only successful for AXB-loaded NLC. The second strategy was the addition of a cationic lipid (DDAB or DOTAP at 0.3%, w/v) to increase the retention time on the ocular surface. A second round of PK studies (PK-II) gave good results for cationic AXB-loaded NLC at 2mM.

The highly concentrated NLC formulation could thus be a potential topical treatment for diabetic retinopathy.

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0.005% and 1.87 ± 0.05%, respectively, which corresponded to encapsulation efficiencies of 18.75 ± 0.19% and 72.14 ± 2.10%, respectively. These values appear significantly higher in comparison to those of Fer loading in the same types of SLMs [4].

The dissolution studies of Fer and Fer-Fer-Me and the release studies of Fer-Fer-Me from the SLMs were performed at 37°C in a mixture of water and methanol (70:30 v/v). Fer-Fer-Me was characterized by a very poor dissolution rate, being solubilized about the 8% of the total raw powder amount after six hours of incubation. The tristearin based SLMs showed a release pattern characterized by a burst effect of about 10% of the incorporated Fer-Fer-Me, followed by a relatively slow release (about 30% of encapsulated prodrug released within 6 h). The stearic acid based SLMs, despite the relatively high encapsulation efficiency, showed a Fer-Fer-Me release pattern characterized by a burst effect of about 50%, followed by a relatively fast release allowing to obtain the dissolution of more than 80% of the loaded prodrug within 6 hours of incubation.

The SLMs based on stearic acid were therefore characterized not only by a satisfactory encapsulation efficiency but also by their ability to induce a fast dissolution of Fer-Fer-Me in comparison to the raw powder. Thus, these microparticles were selected for nasal administration of the prodrug, in order to verify its potential uptake in the CNS. Fer and Fer-Fer-Me were firstly administered by intravenous route (1 mg/kg) in order to evaluate and compare their pharmacokinetic profile in the bloodstream and cerebrospinal fluid (CSF) of rats; then Fer-Fer-Me loaded in SLMs was nasally administered (1 mg/kg) and the pharmacokinetic profiles obtained in the bloodstream and CSF of rats were compared with those obtained by the nasal administration of Fer (1 mg/kg).

The bloodstream half-life of intravenously administered Fer was 20.3 ± 1.3 minutes, with an area under concentration/time curve (AUC) of 244 ± 13 $\mu g \cdot mL^{-1} \cdot min$. The CSF AUC value of the Fer profile (which decreased to zero within 120 min) was 3.3 ± 0.3 $\mu g \cdot mL^{-1} \cdot min$. The half-life in the bloodstream of intravenously administered Fer-Fer-Me was 18.0 ± 1.9 minutes; moreover, relevant amounts of Fer-Me and Fer derived by the hydrolysis of the prodrug were evidenced. The AUC value of the Fer-Fer-Me profile in CSF of rats was 9.8 ± 0.5 $\mu g \cdot mL^{-1} \cdot min$. These results indicate that both Fer and its prodrug Fer-Fer-Me evidence the aptitude to permeate in the CNS of rats from the bloodstream.

Following its nasal administration, Fer was detected during time both in the bloodstream and CSF of rats. The AUC value in the bloodstream was 99.1 ± 4.8 $\mu g \cdot mL^{-1} \cdot min$, allowing to calculate a nasal bioavailability surprisingly high, being its value 40.5 ± 2.8 %. This result seems to confirm the marked ability of Fer to permeate across the physiologic barriers, as evidenced by its aptitude to permeate in the CNS from the bloodstream. The AUC value of the Fer profile in CSF of rats was 5.16 ± 0.2 $\mu g \cdot mL^{-1} \cdot min$, slightly higher than that obtained by intravenous administration of Fer but lower than that of the prodrug Fer-Fer-Me intravenously administered at the same dose.

The nasal administration of Fer-Fer-Me (1 mg/kg) as water suspension did not allow to obtain its detection neither in the bloodstream, nor in the CSF of rats, similarly as its hydrolysis products. This behavior can be attribute to the very low dissolution rate of Fer-Fer-Me in aqueous environments. On the other hand, the nasal administration of a same dose of this prodrug encapsulated in stearic acid based SLMs allowed its detection in both bloodstream and CSF of rats. The profiles of Fer-Fer-Me and its hydrolysis products in the bloodstream suggest that the nasal bioavailability ofthe prodrug is relatively high. Indeed, the sum of the AUC values corresponding to the profiles of all compounds detected in the bloodstream, following intravenous and nasal administration were 560 ± 20 $\mu g \cdot mL^{-1} \cdot min$ and 348 ± 16 $\mu g \cdot mL^{-1} \cdot min$, respectively. Concerning the CSF, the AUC value of the Fer-Fer-Me profile was 108.5 ± 3.9 $\mu g \cdot mL^{-1} \cdot min$, *i.e.* about 20 times higher than that obtained by nasal administration of a same dose of Fer, or about 10 time higher than that obtained by the intravenous administration of the prodrug itself; moreover, at 120 minutes Fer-Fer-Me was still quantifiable.

Overall, the results indicate that the nasal administration of the stearic acid SLMs loaded with Fer-Fer-Me is sensibly efficacious in the prodrug brain targeting, by enhancing both amounts and permanence in the rat CSF, in comparison to Fer. This formulation appears therefore promising for the treatment of neurodegenerative disorders, taking into account the antioxidant and anti-inflammatory activities of the prodrug itself and its ability to be hydrolyzed in central environments to Fer and Fer-Me, which are both known to induce neuroprotective effects [4].

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