Università degli Studi di Palermo

DIPARTIMENTO DI SCIENZE E TECNOLOGIE BIOLOGICHE CHIMICHE E FARMACEUTICHE (STEBICEF)

CRS Italy Local Chapter

October
5 to 7, 2023

Palermo
ITALY

NEXT GENERATION BIOMATERIALS AND CARRIERS: NEW TOOLS FOR DRUG DELIVERY AND THERAPY

CRS ITALY WORKSHOP

Program

Day one Thursday October 5, 2023 (Half Day)
11.00 – 14.00 Welcome and Registration

12.00 – 13.00 Presession (Chair: Prof Silvia Franzè and Prof Michele Schlich) **"Challenges in the preparation of grant proposals: from original ideas to self-assessment"**
(dedicated to young scientists < 35 y.o.):
Prof. Gianfranco Pasut - University of Padua (Italy)

14.00 – 15.10 Session I (Chair: Prof Gennara Cavallaro and Prof Gianfranco Pasut) **"Advanced applications for cancer treatments: natural and synthetic biopolymers for innovative therapies".**
Prof Julien Nicolas, Universitè Paris-Saclay (France), Drug-Initiated Synthesis of Polymer Prodrugs for Anticancer Therapy (35 min)
Prof Nicola Tirelli, IIT Genova (Italy), Hyaluronic acid - a critical review of its interactions with cell surface receptors, and the implications on targeted drug delivery (35 min)

15.10 – 15.40 Coffe Break

15.40 – 17.00 Session II (Chair: Prof Gennara Cavallaro and Prof Gianfranco Pasut) **"Advanced applications for cancer treatments: natural and synthetic biopolymers for innovative therapies".**
Dr Chris Thomas, Lipoid (Germany), Phospholipids in innovative pharmaceutical applications (20 min)
Prof Anna Scomparin, University of Turin (Italy), Modulating glioblastoma microenvironment with cyclodextrin-based nanomedicines (20 min)
Dr Alessio Malfanti, University of Padua (Italy), Local Delivery of STING-Hyaluronic Acid Conjugate Primes Robust Anti-Glioblastoma Immune Response (20 min)
Dr Ortensia Parisi, University of Calabria (Italy), Theranostic Molecularly Imprinted Nanoparticles for Gefitinib Controlled Release (20 min)

17.00 – 18.00 Poster Session I

Day two Friday October 6, 2023 - (Full Day)

08.30 – 10.20 Session III (Chair: Prof Emanuela Craparo and Prof Paolo Caliceti) **"Overcoming physiological barriers in the treatment of chronic inflammation-based diseases".**

Prof Giovanna Lollo, University Lyon 1 (France), Oral nanocomposites for precision medicine (35 min)

Prof Massimo Conese, University of Foggia (Italy), Nanoparticle-based drug delivery systems for dampening inflammation/oxidative stress and enhancing mucopenetration in chronic respiratory diseases (35 min)

Dr Marialisa Pia Dimmito, University of Chieti (Italy), Lipidated peptide hydrogel: a platform for the delivery of inflammatory bowel diseases therapeutics (20 min)

Dr Saliha Mountaharrick, University of Milan (Italy), Oral Delivery System Leveraging Guar Gum as a Microbially Degradable Component for Colonic Release (20 min)

10.20 – 10.50 Coffee Break

10.50 – 12.40 Session IV (Chair: Prof Rossella Dorati and Prof Pasquale Del Gaudio) **"Protein and nucleic acid delivery: approaches for cell engineering and disease treatment".**

Prof Kevin Braeckmans, Ghent University (Belgium), Delivering effector molecules in cells in vitro and ex vivo by photoporation (35 min)

Prof Virginia Arechavala-Gomeza, Biocruces Bizkaia Health Research Institute (Spain), Nucleic acid therapeutics may change the way we treat disease, but not until we solve the delivery problem (35 min)

Dr Giulia Anderluzzi, University of Milan (Italy), Lipopolyplexes for DNA delivery: understanding the impact of cationic polymer on cytotoxicity and transfection (20 min)

Dr Enrica Chiesa, University of Pavia (Italy), Systematic approach for the optimization of Lipid Nanoparticles for siRNA delivery in lung diseases (20 min)

12.40 – 14.20 Lunch Break

(Discussion and Poster Session II)

14.20 – 15.50 Session V (Chair: Prof Silvia Pescina and Prof Calogero Fiorica) **"Advanced therapeutics in precision medicine applications".**

Prof Michael Malkoch, KTH Royal Institute of Technology, Stockholm (Sweden), Dendritic hydrogels as regenerative and antibacterial materials (35 min)

Prof Maurizio Pesce, Centro Cardiologico Monzino (IRCCS), Milan (Italy), Tailored targeting of mechano-sensitive in human cardiac fibroblasts for reduction of fibrosis and heart failure (35 min)

Dr Nicolò Mauro, University of Palermo (Italy), The Emerging Role of Carbon Nanodots in Precision Nanomedicine and Composites Biomaterials (20 min)

15.50 – 16.40 Poster Session II

16.40 – 18.15 Session VI (Chair: Prof Roberta Cavalli and Prof Francesca Mastrotto) **"Innovative Drug Delivery Carriers, Formulation and Characterization".**

Prof Elena Del Favero, University of Milan (Italy), Structural characterization of biomaterials and carriers at the nanoscale (35 min)

Dr Roberto Santoliquido, Alfatest srl (Italy), Why using microfluidics for nanoparticle synthesis offers you so many advantages (20 min)

Dr Giorgia Adamo, CNR Palermo (Italy), Harnessing Nature's Secrets: Microalgal-Derived Extracellular Vesicles as Bio-Based delivery system for next-level Pharmaceutical and Cosmetic applications (20 min)

Prof Rosario Pignatello, University of Catania (Italy), Self-nanoemulsifying drug delivery systems (SNEDDS) for ocular delivery of natural compounds with SIRT-1 activator activity (20 min).

20.00 Social Dinner

Istituto professionale Pietro Piazza – Corso dei Mille 181

Day Three Saturday October 7, 2023 (Half-Day)

09.00 – 10.30 Session VII: (Chair: Dr Chiara Bastiancich and Prof Francesco Puoci) **"Spin-off ideas: from academic research to the technology transfer".**

(Special Session organized by the CRS Italy Young Scientists)

Prof Julien Nicolas, Universitè Paris-Saclay (France), Unlocking the full potential of highly potent anticancer drugs (30 min)

Prof Kevin Braeckmans, Ghent University (Belgium), From research to spin-off: a troublesome road or an exciting adventure? (30 min)

Round table

10.30 – 11.00 Coffee Break

11.00 – 12.50 Session VIII, (Chair: Prof Barbara Stella and Prof Nunzio Denora) **"Innovations in micro-engineering tools: from diagnosis to treatment".**

Prof Ben M. Maoz, Tel Aviv University (Israel), Organs-on-a- Chip: A new told for studying human physiology (35 min)

Prof Paolo A. Netti, IIT Naples (Italy), On-chip simulation of the efficacy and safety of novel drug delivery approaches: achievements and challenges (35 min)

Dr Rosa Maria Iacobazzi (Italy), Microfluidic Production of Biomimetic Liposomes for personalized therapy of metastatic melanoma (20 min)

Dr Ovidio Catanzano, CNR Naples (Italy), Nano-in-nanofibers advanced dressing for spatio-temporal delivery of growth factors (GFs) in chronic wound healing (20 min)

ABSTRACT BOOK

Thursday October 5, 2023
Oral Presentations

Drug-Initiated Synthesis of Polymer Prodrugs for Anticancer Therapy

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ABSTRACT

We report on the design of a new class of polymer prodrug nanocarriers by using the "*drug-initiated*" method,[1] which consists in the controlled growth of vinyl polymers from anticancer drug-bearing initiators to prepare well-defined and high drug content polymer prodrug nanoparticles with in vitro and in vivo anticancer activity.[2] This method is robust and versatile as it was applied to different anticancer drugs, different polymers and different drug/polymer linkers to adjust the drug release kinetics and thus the cytotoxicity.[3,4] This approach was further developed to yield heterotelechelic polymer prodrugs for imaging and combination therapy.[5] We also designed well-defined hydrophilic polymer prodrugs suitable for the subcutaneous administration of vesicant/irritant anticancer drugs in order to circumvent the limitations and drawbacks associated to IV chemotherapy.[6] We also developed efficient synthetic pathways to confer degradability to these polymer prodrugs by means of radical ring-opening polymerization.[7,8]

References:

1. Nicolas, J. ***Chem. Mater.*** **2016***, 28*, 1591
2. Harrisson, S.; Nicolas, J.; Maksimenko, A.; Bui, D. T.; Mougin, J.; Couvreur, P. ***Angew. Chem., Int. Ed.*** **2013**, *52*, 1678
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5. Vinciguerra, D.; Denis, S.; Mougin, J.; Jacobs, M.; Guillaneuf, Y.; Mura, S.; Couvreur, P.; Nicolas, J. *J. Control. Release* **2018**, *286*, 485
6. Bordat, A.; Boissenot, T.; Ibrahim, N.; Ferrere, M.; Levêque, M.; Potiron, L.; Denis, S.; Garcia-Argote, S.; Carvalho, O.; Abadie, J.; Cailleau, C.; Pieters, G.; Tsapis, N.; Nicolas, J. *J. Am. Chem. Soc.* **2022**, *144*, 18844
7. Lages, M.; Pesenti, T.; Zhu, C.; Le, D.; Mougin, J.; Guillaneuf, Y.; Nicolas, J. ***Chem. Sci.*** **2023, *14*, 3311**
8. Bossion, A.; Zhu, C.; Guerassimoff, L.; Mougin, J.; Nicolas, J. *Nature Commun.* **2022**, *13*, 2873

Hyaluronic acid - a critical review of its interactions with cell surface receptors, and the implications on targeted drug delivery

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ABSTRACT

Hyaluronic acid (HA) is a naturally occurring biomolecule, which has found widespread clinical use as a biomaterial. It is also an attractive component of (nano)systems designed to deliver drugs in a targeted fashion: HA has virtually no toxicity, is degradable, is easily chemically functionalized, and, most importantly, can allow a systemically circulating structure A) to accumulate in solid tumours and B) to be internalized in a receptor-mediated fashion. Facing all these positive features, relatively little is known at a detailed, mechanistic level. This talk will focus on point B, specifically discussing the molecular controlling factors of the interactions between HA and its most widespread receptor, CD44.

PHOSPHOLIPIDS IN INNOVATIVE PHARMACEUTICAL APPLICATIONS

Dr Chris Thomas, Lipoid

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ABSTRACT

Phospholipids are well established excipients for pharmaceutical applications. They are used in many types of formulations, like fat emulsions, mixed micelles, suspensions and liposomal preparations for any administration route. They are natural compounds and effective alternatives to synthetic, unnatural emulsifiers, like polysorbates, polyoxyethylene castor oil derivatives and sucrose fatty acid esters.

Phospholipids are phosphorus-containing lipids, surface-active, amphiphilic ("both loving") molecules, which comprise a polar, hydrophilic headgroup and a lipophilic tail connected by a glycerol moiety. Arranged as a lipid bilayer, phospholipids constitute a major structural and functional component of all cell membranes. Because of their amphiphilic character, phospholipids act as emulsifiers and they can form lipid micelles or bilayers (e.g., liposomes) depending on their chemical structure.

When suspended in water, most phospholipids form lipid bilayers and/or closed lipid vesicles,

These vesicles consist of an aqueous core surrounded by one or several phospholipid bilayers. Liposomes are suitable to encapsulate water soluble drugs and to solubilize poorly water-soluble drugs for oral, topical, and parenteral use.

Natural phospholipid excipients are defined as phospholipids isolated from natural sources like e.g. soybean and sunflower seed. They may be further converted to saturated phospholipids by means of hydrogenation or to molecular species with any type of headgroup and fatty acid chain.

Phospholipids are multifunctional excipients that can contribute many functions to formulations

for any route of administration (for example parenteral, oral, dermal, ophthalmic, pulmonary, etc.).

Technical use of phospholipids:

• Emulsifier

• Solubilizer (e.g., in mixed micelles)

• Wetting agent (suspending aid)

• Component of colloidal structures (liposomes, micelles)

Functional use of phospholipids:

• Increase of therapeutic index

• Bioavailability enhancer

- Modification of drug release (depot formulation)
- Reduction of toxicity
- Penetration enhancer
- Moisturizer
- Texturizer

While phospholipids are well known in various commercial emulsion and liposome-based products, more recently, they become essential part in several other sophisticated drug delivery technologies such as in vaccinations and depot formulations.

Modulating glioblastoma microenvironment with cyclodextrin-based nanomedicines

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ABSTRACT

Glioblastoma is the most aggressive of brain cancers and currently its treatment includes surgery, radiation, anti-angiogenic therapy and chemotherapy. Temozolomide is the gold standard drug but a number of patients do not respond due to chemoresistance issues. In the last few years, with the general recognition of the efficacy of immunotherapy in cancer treatment, and the concomitant identification of lymphatic vessels in the brain, immune-checkpoint modulators, CAR T cells and vaccines have been exploited for glioblastoma therapy but has yet to show improved survival on this devastating disease. Recently, the tyrosine kinase inhibitor ibrutinib has been shown to be active in inhibiting glioblastoma, targeting tumorigenic glioma stem cells [1]. The synergistic effect of two drugs with different mechanisms of action represents a great therapeutic potential. In order to fully exploit this strategy, we developed a nano-sized polymeric system, based on β-cyclodextrin (β-CD), a natural cyclic oligosaccharide. The repeating unit of β-CD were reacted with different crosslinking agents, to obtain linear, branched or hyper-crosslinked polymers. The latter, defined as nanosponges (NS), are nano-sized (~250 nm) polymers with multiple domains, i.e. hydrophobic β-CD cavities and hydrophilic nanochannels, that can be exploited to load active molecules [2]. These possess high encapsulation efficacy for temozolomide (92.08%, with a loading capacity of 7.56%) and ibrutinib (62.60%, with a loading capacity of 5.35%). The formulation retains the in vitro cytotoxicity of the drugs on a rat undifferentiated malignant glioma cell line (F98 cells). Furthermore, the NS can be conjugated to the inducible T cell co-stimulator (ICOS) protein, which has been demonstrated to have several implications on cancer progression [3]. ICOS-decorated NS loaded with temozolomide and ibrutinib were able to inhibit F98 cells migration. β-CD-based branched polymers were modified to introduce positively-charged amino groups, and are able to form electrostatic complexes with oligonucleotides. Polyplexes carrying siRNA against the Serum- and Glucocorticoid-regulated Kinase (SGK1) were able to silence the targeted gene (up to 80%), which is often upregulated in glioma stem cells, and represent a promising therapeutic target [4]. The newly developed β-CD-based polymers can be easily tailored to develop several types of nanomedicines to improve the efficacy of anticancer drugs, oligonucleotides and immunotherapeutic agents for personalized therapy.

References:

[1] Sala L. *et al.*, *Frontiers in Molecular Neuroscience* **2019,** *12*.

[2] Trotta F. *et al.*, *Expert Opin Drug Deliv.* **2014**, 11(6):931-41.

[3] Clemente N. *et al., J Control Release.* **2020**;320:112-124.

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Local Delivery of STING-Hyaluronic Acid Conjugate Primes Robust Anti-Glioblastoma Immune Response

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ABSTRACT

Glioblastoma (GBM) is the most aggressive brain tumor with a highly immunosuppressive tumor immune microenvironment (TIME). Currently, GBM remains largely refractory to immunotherapeutic approaches [1]. In this work, we investigated the use of STimulator of INterferon Genes (STING) pathway as an effective means to remodel the GBM TIME. Leveraging our experience in the use of Hyaluronic Acid (HA) conjugates for local treatment of GBM [2,3], we hypothesized that the conjugation of a non-nucleotide STING agonist (MSA2) to HA could impact the GBM immunity cycle by eliciting combined local innate and adaptative immune responses. HA was selected for its high affinity toward the CD44 receptor, expressed in GBM and immune cell surfaces and its efficient drainage into the brain lymphatic system. MSA2 was conjugated to HA using a pH-sensitive linker and was chemically-physically characterized using ^{1}HNMR, UV-Vis and Dynamic Light Scattering. HA-MSA2 display a drug loading of 1.4% w/w, a size of ≈12 nm and a negative zeta potential (-25 mV). In vitro studies show an upregulation of type I IFN signalling pathways (e.g., IFNβ) mediated by HA-MSA2 over the free drug in murine immune (JAWSII dendritic cells, J774 macrophages and BV-2 microglia) and GBM (GL261 and SB28) cells. Interestingly, HA-MSA2 elicited a simultaneous cancer cell-intrinsic mechanism of innate immunity and promoted immunogenic cell death of GBM cells. Next, the activity of HA-MSA2 has been assessed in vivo by a single local administration of HA-MSA2 in a highly "cold" orthotopic SB28 murine model. We observed an extension of survival of SB28 GBM tumor bearing mice for mice treated with HA-MSA2 (6 days over the control). The analysis of the TIME showed a profound shift in the GBM immune landscape after HA-MSA2 treatment, allowing a large infiltration of innate and adaptative immune cells. Overall, we introduced a state-of-the-art biodegradable polymer platform to deliver a potent STING agonist MSA2. This study demonstrates a significant GBM TIME remodelling through STING activation induced by HA-MSA2 conjugate.

References:

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Theranostic Molecularly Imprinted Nanoparticles for Gefitinib Controlled Release

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ABSTRACT

Lung cancer consists of a group of molecularly and histologically heterogeneous subtypes, including small-cell lung carcinoma and non-small-cell lung carcinoma (NSCLC) [1], and represents the most common cause of cancer death. The epidermal growth factor receptor (EGFR) has emerged as a promising therapeutic target because of its overexpression in NSCLC and Gefitinib (GEF), which is a first-generation EGFR tyrosine kinase inhibitor, is the first example of molecularly targeted agent for the treatment of NSCLCs [2]. However, its low solubility and poor bioavailability require a high dose to get the therapeutic effect leading to additional adverse reactions and an acquired resistance in patients with NSCLC after one-year treatment [3]. In this context, the development of a delivery system able to control GEF release could represent an effective strategy to reduce the required dose and, thus, the side effects.

Theranostic agents are attracting considerable research attention due to their ability to combine at the same time cancer diagnosis with therapy. Therefore, we reported the development of a novel theranostic nanosystem able to combine the drug-controlled release ability of Molecularly Imprinted Polymers (MIPs) with the detection properties of a fluorescent monomer for the real-time evaluation of the therapeutic response to the treatment and to enhance both the efficacy and safety of the anticancer agent. For this purpose, a fluorescein-modified monomer was included in the reaction mixture to provide fluorescent properties to the material, enabling its use as diagnostic agents or for cancer staging (Fig. 1).

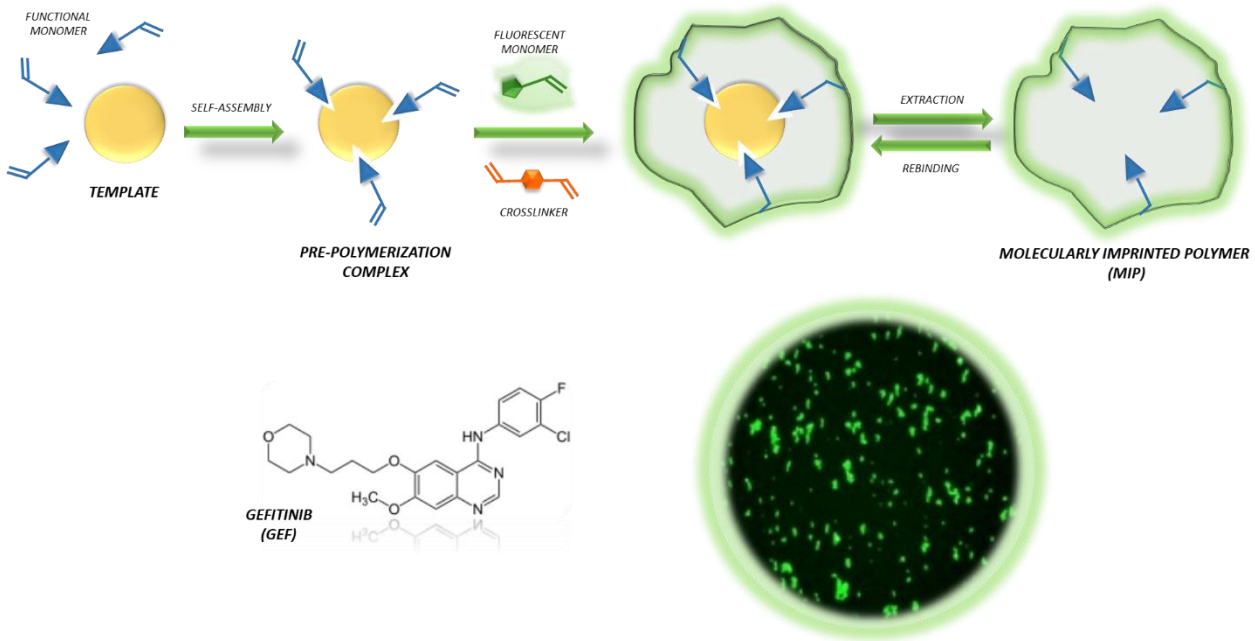

Figure 1. *Schematic representation of GEF imprinted nanoparticles synthesis.*

Particles were characterized in terms of size, shape, protein adsorption measurement, swelling behavior, and fluorescent properties. *In vitro* release studies as well as binding experiments at different times and concentrations in the presence of the template molecule and a structural analogue were carried out. In addition, cytotoxicity and hemolysis studies were performed.

The obtained results confirmed the ability of the imprinted nanoparticles to bind in a specific and selective way the targeted molecule, which was released in a controlled manner, and their biocompatibility, therefore, representing a promising tool for cancer nanomedicine.

Next steps will involve the functionalization of the polymer surface with biomolecules able to address the attachment of the nanoparticles to specific cell populations (NSCLC), promoting drug accumulation at the pathological site.

References:

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Friday October 6, 2023
Oral Presentations

Oral nanocomposites for precision medicine

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ABSTRACT

Oral delivery is considered the preferred route of administration encompassing over 50% of FDA approvals, and allowing for localized or systemic delivery at gastrointestinal (GI) level [1]. However, the therapeutic efficacy of several hydrophobic and hydrophilic drugs and biologics is hampered by their solubility in GI fluids and/or permeability, and by their chemical/enzymatic stability. Nanosystems (NPs) has been designed to overcome these drawbacks, but they undergo destabilization caused by the presence of gastric acid, bile salts, and lipases [2]. Furthermore, NPs have to deal with high instability, rapid clearance and very poor predictability of their fate once ingested. The creation of hybrid systems improved NPs stability and permeability in the gastrointestinal tract [3]. Hybrid systems can be defined as nanocomposites when they possess at least one nano-scaled phase, dispersed in a matrix. In this presentation, the rational design of nanocomposites made of lipid nanosystems embedded into a polymeric matrix is presented [4]. These systems are aimed to target inflammatory bowel diseases (IBD) following oral administration. Overall, the research here presented at the interface of pharmaceutical technology and biology show how nanotechnologies may improve the treatments of IBD and as future perspective can be used for oral delivery of biologics.

References:

1. Andretto V. et al., Drug Deliv Transl Res 2021
2. He H. et al., Acta Pharm Sin B 2019
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Nanoparticle-based drug delivery systems for dampening inflammation/oxidative stress and enhancing mucopenetration in chronic respiratory diseases

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ABSTRACT

Chronic respiratory diseases, i.e. cystic fibrosis (CF), allergic asthma and chronic obstructive pulmonary disease (COPD, whose pathological hallmarks are oxidative stress, persistent inflammation and tenacious viscous mucus, need novel therapeutic tools and pharmaceutical agents. In the effort to develop drug delivery systems able to avoid both the adhesion trapping to mucin network and the steric inhibition by the dense mucin fibers, we have evaluated whether Small Unilamellar Vesicles (SUVs) (<100 nm), with a polymeric surface modifier such as PEG lipids, were able to overcome the sputum obtained from COPD outpatients [1]. The penetration studies showed that PEG-SUVs were the most mucus-penetrating vesicles after 27 h. We have previously demonstrated the ability in reducing oxidative stress and inflammation by the red grape seed extract (GSE), encapsulated in biocompatible solid lipid nanoparticles (SLNs), in airway epithelial cells *in vitro* [2]. Thus, we tested magneto-sensitive iron oxide containing SLNs (mSLNs) based on Gelucire® 50/13 [3] on mucus samples derived from different sources. In the presence of a magnetic field, mSLNs were more permeable in porcine gastric mucus and less in COPD outpatient sputum, while high and low secretions obtained from COPD patients during their admission in the intensive care unit are still difficult to overcome. Overall, these results highlight the possibility to employ different strategies to overcome the mucus barrier in the respiratory tract in pathological conditions, allowing to target safely epithelial cells with the ultimate aim to deliver therapeutic molecules.

References:

[1] De Leo, V.; Ruscigno, S.; Trapani, A.; Di Gioia, S.; Milano, F.; Mandracchia, D.; Comparelli, R.; Castellani, S.; Agostiano, A.; Trapani, G.; Catucci, L.; Conese, M., Preparation of drug-loaded small unilamellar liposomes and evaluation of their potential for the treatment of chronic respiratory diseases. Int J Pharm 2018, 545, (1-2), 378-388.

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LIPIDATED PEPTIDE HYDROGELS: A PLATFORM FOR THE DELIVERY OF INFLAMMATORY BOWEL DISEASES THERAPEUTICS

Marilisa Pia Dimmito [*1], Antonio Di Stefano[1,2], Lisa Marinelli[1,2], Giorgia Carraro[3], Barbara Albertini[4], Alessandra Rapino[4], Eleonora Chiara Toto[1], Ivana Cacciatore[1,2]

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ABSTRACT

Hydrogel biomaterials are acquiring much interest for their tunable physical properties, flexibility, encapsulation ability, water-holding capacity, and controllable degradability. Lipopeptides are a class of antimicrobial peptides widely implied in the treatment of infectious diseases with a low tendency to develop bacterial resistance [1]. Depending on external stimuli, lipopeptides possess an outstanding tendency to aggregate into defined supramolecular structures, quite suitable for biomedical applications. Lipopeptides are also implied in the treatment of Inflammatory Bowel Diseases (IBD), often provoked by intestinal microbiota imbalance, whose main symptoms include inflammation, severe pain, and susceptibility to infections [2]. Among the discovered AMPs, a small peptide with sequence Ser-Asn-Ala (SNA) showed moderate antibacterial activity against both Gram-positive and negative bacteria [3]. In this work SNA structure was modified to overcome its shortcomings through lipidation; this modification led to a series of SNA analogues, also containing different combinations of D-, and L-aminoacids. This strategy is aimed to endorse SNA with self-assembly capability allowing it to arrange into ordered supramolecular structures and provide a platform for drug delivery [4]. To explore the suitability of this platform as a drug delivery system, budesonide (BUD) was loaded as a prototype of an anti-inflammatory drug.

Fig. 1. Lipopeptide-based hydrogels.

MPD*h*_02-09 hydrogels (**Fig. 1**), were prepared and characterized in terms of Critical Gelation Concentration (CGC), surface tension (pendant drop method), mechanical features through rheological tests, morphological properties by Atomic Force Microscope (AFM) measurements and *in vitro* drug release. *In vitro* biological assays were performed to assess the safety and antimicrobial activity. Results showed that **MPD*h*_02-09** have a CGC and surface tension ranging from 4-8 mM and from 46 to 65 mN/m, respectively. Morphological analysis revealed a nanofibrillar network. Moreover, **MPD*h*_02-09** showed a solid-like behavior with viscoelasticity and stiffness depending on the concentration with the storage modulus G′ higher than the loss modulus G″. Good antimicrobial activity *vs H. pylori*, *C. difficile*, and *F. nucleatum* (MIC values from 2 to 128 µg/mL) and no toxicity – at the concentrations ranging from 1 to 100 µM – were observed.

MPD*h* mucoadhesive gels can represent a valid platform to explore a synergistic effect of "sticky steroid" BUD and lipopeptide as a therapeutic option for eosinophilic oesophagitis (EoE) as well as IBD.

References:

[1] Hong, J., et al. Eur. Food Res. Technol., 2015, 240, 327-333.

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Oral Delivery System Leveraging Guar Gum as a Microbially Degradable Component for Colonic Release

S. Moutaharrik [*1], G. Meroni[2], A. Foppoli[1], M. Cerea[1], L. Palugan[1], A. Gazzaniga[1], P.A. Martino[2], A. Maroni[1]

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ABSTRACT

Naturally occurring polysaccharides that are degraded by the resident microbiota have largely been exploited for oral colon delivery. However, their hydrophilic nature may negatively impact on the relevant targeting effectiveness. In order to overcome this drawback, a variety of strategies have been attempted, mainly including chemical derivatization and combination with insoluble polymers such as ethyl cellulose, polymethacrylates or cellulose acetate [Maroni, 2013]. Additionally, natural polysaccharides have been combined with enteric soluble polymers to improve pH-dependent colon delivery systems by tackling the release failure issue often observed with merely pH-dependent formulations [Ibekwe, 2008; Maroni, 2017; Moutaharrik, 2021; Moutaharrik, 2023]. In the present work, a double-coated delivery system leveraging intestinal microbiota, pH and transit time was proposed to achieve more reliable colonic release. This system consisted of a tablet core, an inner layer of swellable hydroxypropyl methylcellulose (HPMC), and an outer coating composed of Eudragit® S and guar gum (7:3 solid weight ratio), a linear galactomannan derived from guar seeds. Both layers were applied by spray-coating in a fluid bed equipment of tablet cores (4 mm of diameter) containing a tracer drug (acetaminophen). In the Eudragit® S/guar gum coating formulation, guar gum was used either as an aqueous solution or a hydro-alcoholic dispersion. When the double-coated systems were tested in 0.1 N HCl followed by phosphate buffer pH 7.4, guar gum was demonstrated not to impair the barrier properties of the enteric film when incorporated in dispersed form. Lag phases of consistent duration were imparted by the HPMC layer and synergistically extended by the overlaid Eudragit® S/guar gum coating. The delivery systems were also evaluated in simulated colonic fluid (SCF) containing fecal bacteria from a Crohn's disease patient. SCF was obtained from an adequate culture medium by an experimental procedure purposely adopted to enable multiple tests from a single sampling and processing run, thus reducing the time, costs and complexity involved and enhancing replicability. In such a fluid, release was notably faster than in control culture medium (Figure 1). The ability of guar gum to trigger the release of the loaded drug in the colon environment was thereby demonstrated, along with its potential for enhancing the targeting properties of a colon delivery system based on a combined formulation approach.

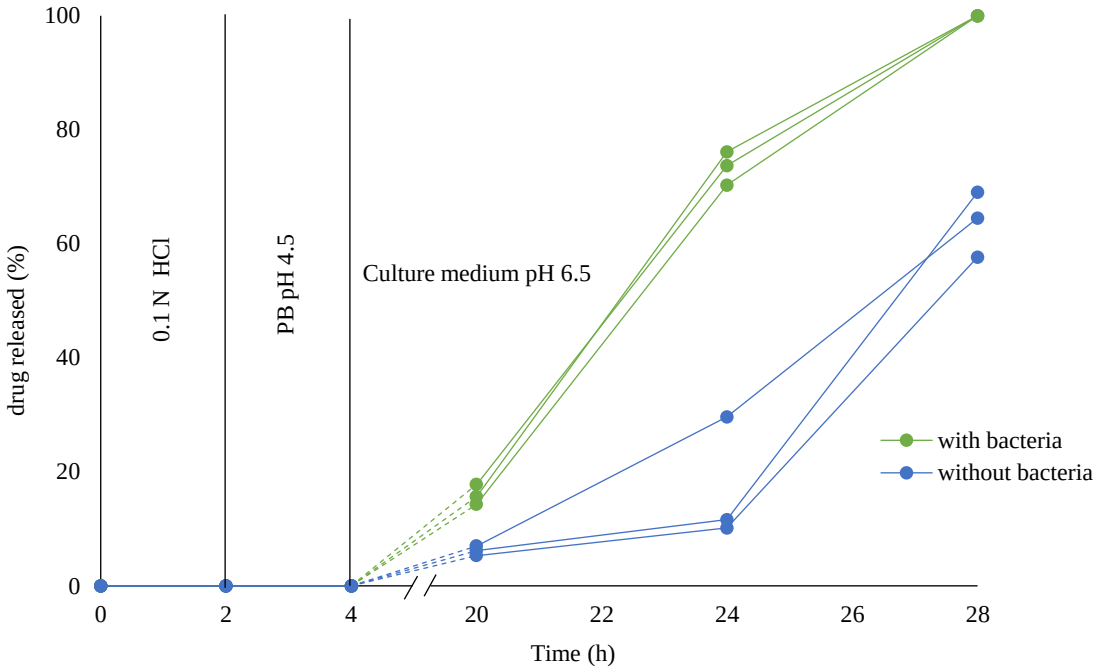

Figure 1: acetaminophen release profiles from double-coated systems (14 mg/cm^2 of Eudragit® S) upon exposure to 0.1 N HCl for 2 h, phosphate buffer pH 4.5 for 2 h and then culture medium inoculated with fecal bacteria or culture medium as such.

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Delivering effector molecules in cells in vitro and ex vivo by photoporation

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ABSTRACT

Delivery of bioactive compounds, such as proteins and nucleic acids, into cells in vitro or ex vivo is a generic requirement for many applications in the life sciences, such as for the engineering of therapeutic cells. Physical delivery methods are attractive in this context as they are well-controlled, and can accommodate a broad variety of effector molecules and cell types. Photoporation is such a recently developed physical delivery technology which combines laser stimulation with photothermal nanoparticles. Localized thermal effects upon laser irradiation can create pores in the cell membrane, allowing the influx of external molecules in cells. Importantly, photoporation is very gentle to cells, resulting in excellent cell viability and preservation of a cell's phenotype and functionality. In this presentation I will give an overview of the most notable work that we performed on photoporation as a next-generation transfection technology in the past decade.

Nucleic acid therapeutics may change the way we treat disease, but not until we solve the delivery problem

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ABSTRACT

New drugs, based on nucleic acids have the potential of becoming a new paradigm in pharmacology: they should be much easier to "design", as they could target very specifically to their targets. However, there are many hurdles that need to be considered before those drugs become mainstream and the main one is their deficient delivery to target tissues. Prof. Arechavala-Gomeza has participated in the development of some of these drugs and will provide the audience with a summary of how they work and what is still needed to make them a viable therapeutic alternative.

Lipopolyplexes for DNA delivery: understanding the impact of cationic polymer on cytotoxicity and transfection

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ABSTRACT

Lipopolyplexes (LPP), namely ternary complexes of biocompatible cationic polymers, nucleic acid and liposomes, represent an attractive though poorly investigated alternative to lipoplexes for gene delivery. Poly-L-lysine (PLL), polyethyleneimine (PEI), spermidine, spermine and protamine sulfate are the most used polymers for LPP preparation[1]. Nevertheless, a systematic study of the polymer nature on nucleic acid complexation and transfection efficiency is missing. Here, we prepared three LPP containing three cationic polymers (Chitosan, PLL and PEI) and 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) based liposomes to complex a pDNA encoding the green florescence protein (EGFP). Briefly, pDNA encoding EGFP was complexed with a solution of chitosan, PLL and PEI in PBS 50mM pH 4.5 at different polymer/DNA mass ratios. Selected polyplexes were further complexed with DPPC:DOPE:Chol liposomes (55:35:10 mol%, in PBS 50 mM pH 7.4) at 0.5 or 0.2mM. All formulations were characterized in terms of hydrodynamic size, polydispersity index (PDI) and zeta potential by dynamic light scattering. Selected formulations progressed into in vitro studies. Cytotoxicity was assessed by an MTT assay on HEK293 human embryonic kidney cells and primary human adipocytes while the transfection efficiency was quantified in HeLa human epithelial cells by GFP detection. A pDNA/DOTAP cationic lipoplex was used as positive control.

We found that optimal polymer/DNA mass ratios were 10 for chitosan and PLL polyplexes and 13 for PEI polyplexes (corresponding to a N/P ratio of 15, 23 and 46 respectively). Chitosan, PLL and PEI polyplexes had a particles size of 275±3, 282±7 and 324±40 nm respectively with low PDI. Polyplexes surface charge was strongly positive ranging from +32 to +40 mVolt. The final optimal polyplex/liposome ratio was found to be 7, 24 and 27 for chitosan, PLL and PEI LPP, respectively. Ternary complexes had a mean diameter of 165 nm and PDI<0.2; of note, while chitosan and PLL LPP were positively charged (+13.8 and +8.4 mVolt respectively) - though less than the polyplex, PEI LPP were indeed neutral. In vitro, chitosan LPP were poorly cytotoxic, within the range 100-30 µg/ml, whereas PEI and PLL LPP reduced the cell viability at the highest concentrations. Conversely, PEI LPP induced the highest GFP expression in HeLa cells at 24 h, similar to a DOTAP based lipoplex, while chitosan and PLL LPP mediated gene expression after 48h. Of note, while PEI LPP transfected a lower number of cells with higher GFP signal, chitosan LPP induced the highest number of GFP+ cells at lower fluorescence intensity.

In conclusions, results suggest that the choice of polymer influence the kinetics and the mode of gene expression mediated by these LPP. Further, chitosan is a promising polymer for the design of LPP as it combines low cytotoxic profile with good transfection efficiency, offering a safer alternative to cationic lipid for pDNA delivery.

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Systematic approach for the optimization of Lipid Nanoparticles for siRNA delivery in lung diseases

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ABSTRACT

This study is addressed to silence pathologically overexpressed genes in lung diseases with siRNA. In this view, lipid nanoparticles (LNPs) have currently showed to carry RNA and protect it from the degradation [1]. However, LNPs optimization is required to boost the potency and efficacy of nanomedicines in accordance with the specific pathology.

Here, design of experiments (DoE) approach allowed us to optimize siRNA-LNPs features and the in vitro behaviour, envisaging pulmonary administration. siRNA-loaded LNPs were produced by microfluidics. The effect of lipid composition and preparation conditions on particle size and encapsulation efficiency of siRNA (EE%) were studied by a two-level full factorial DoE. Tested manufacturing conditions and the related values are i) type of PEG-lipid (DSPE-PEG, DMG-PEG), ii) type of ionic lipid (DOTAP or D-Lin-MC3-DMA), iii) concentration of ionic lipid and iv) N/P ratio.

The obtained LNPs were analysed by DLS, and the siRNA content was assessed by SYBR gold assay. The siRNA release from the LNPs and the formulation stability were evaluated. Finally, the lead formulation was tested in vitro for cell uptake and gene knockdown efficiency. 16 experiments were run for DoE. Results showed that the type of pegylated lipid, type of cationic/ionizable lipid, and the interaction between cationic lipid and its concentration affected the LNP size. In parallel, the type of PEGylated lipid, N/P ratio, the interaction between these two factors, and the interaction between cationic lipid and its concentration influenced the EE%. The best formulation was obtained with the formulation based on D-Lin-MC3-DMA (20.4% mol), DMG-PEG2000 (0.6% mol), DSPC (60% mol), and Cholesterol (19%mol). By using an N/P ratio of 4, this siRNA-LNP exhibited a size of 140 nm (PDI=0.21) and an EE% of 87%. siRNA release and stability studies in the simulated interstitial lung fluid complemented the optimal characteristics required for pulmonary formulation. Cell uptake and gene knockdown of the lead siRNA-LNP demonstrated the in vitro performance.

In conclusion siRNA-LNPs were optimized using the DoE approach. The statistical tool pointed out the best formulation containing optimal properties and fostering successful cell uptake and gene knockdown outcomes.

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Dendritic hydrogels as regenerative and antibacterial materials

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ABSTRACT

Dendritic polymers are highly branched macromolecules with large representation of functional groups displayed at surface. Within this family of polymers, flawless dendrimers and polydisperse hyperbranched as well as linear-dendritic block copolymers are well studied as nanocarriers for therapeutic applications. However, the high functional group density of dendritc polymers makes them ideal multifunctional building blocks to generate advanced dendritic networks with biological function. In here, the synthesis and biological evaluation of hydrolytically degradable dendritic hydrogels based on 2,2-bismethylol propionic acid (bis-MPA) will be highlighted. This include antibacterial cationically charged hydrogels that are spontaneously crosslinked through ionic interactions or NHS/amine chemistry as well as neutral hydrogels *via* on-demand UV-initiated thiol-ene click reactions. Hybridization with cellulose nanofibrils or placenta powder as well as the unique biological features of these hydrogels will also be detailed.

Tailored targeting of mechano-sensitive in human cardiac fibroblasts for reduction of fibrosis and heart failure

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ABSTRACT

We recently unraveled the role of mechanically activated YAP/TAZ complex in cardiac fibrosis and maladaptive remodeling of the myocardium. Given the pleiotropic functions of YAP/TAZ in cardiac biology, targeting in the myocardium requires carriers with competence to deliver inhibitors of the transcriptional complex selectively in cardiac fibroblasts and inflammatory cells (where it has a pro-fibrotic/pro-inflammatory role) but not in cardiomyocytes (where it has a pro-survival function). To this aim we elaborated a strategy to engineer nan-theranostic particles able to deliver cargos based on coating with a moiety specific for cellular receptors expressed in the pathologic cells. In the course of the talk, I will expose the basic principles of mechano-therapeutic approaches for cardiovascular pathologies and will present the first experimental proofs-of-concepts.

The Emerging Role of Carbon Nanodots in Precision Nanomedicine and Composites Biomaterials

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ABSTRACT

Carbon nanodots (CDs) are 0-D nanoparticles with diameters of 1-10 nm. CDs have garnered significant attention in the pharmaceutical field due to their environment-sensitive fluorescence imaging contrast properties and suitable photothermal conversion efficacy. They possess several additional advantages such as cost-effectiveness, high water solubility, and biocompatibility [1]. The emission characteristics of CDs make them excellent probes for fluorescence imaging, useful in early disease diagnosis and monitoring. In terms of chemical and photo-stability, biocompatibility, and tunable emission properties, CDs outperform fluorescent organic dyes and nanoparticles with similar emission properties, such as semiconductor quantum dots. Consequently, they are employed to label cancer cells, enabling their detection through flow cytometry and facilitating investigation at the molecular level via fluorescence microscopy. This capability enables a comprehensive understanding of intracellular processes and accurate evaluation of tumor progression. Furthermore, CDs are promising theranostic tools due to their capacity to convert NIR light into heat under the guidance of images, which can be utilized for direct damage to cancer cells through local hyperthermia (42-49°C). Several studies have demonstrated that hyperthermia allows pulsed drug release from CDs, providing remote spatio-temporal control. Remarkably, S-doped CDs selectively induce ROS release in cancer cells, thereby imparting inherent anticancer activity [2]. Consequently, the ability to remotely control CDs using external NIR light and the intrinsic anticancer properties exhibited by S-doped CDs contribute to enhanced pharmacological activity, surpassing the conventional nanomedicine paradigm in which nanomedicines are regarded as EPR-based inert vehicles for drugs.

CDs have also been employed as functional excipients for the development of polyester-based nanoparticles. By incorporating polar CDs, the stability of PLA nanoparticles can be improved, endowing them with fluorescence properties and enhancing the drug loading, ultimately enabling controlled release over time. Similarly, CDs have been utilized in the production of fluorescent and NIR-active PLA scaffolds possessing self-cleaning and self-tracking capabilities. Notably, CDs-containing scaffolds, including electrospun and printed scaffolds, have been developed for the eradication of nosocomial biofilms upon NIR irradiation. Furthermore, they exhibit suitable biodegradability, fluorescent imaging capability, and promote bone regeneration in vitro [3]. Overall, CDs possess

immense potential for the development of photostable biomaterials with diverse pharmaceutical applications, ranging from precision nanomedicine to regenerative medicine.

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Structural characterization of biomaterials and carriers at the nanoscale

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ABSTRACT

Scattering techniques are well suited for studying the physicochemical properties of biomaterials, nanoparticles, and aggregates in solution, meanwhile being largely non-invasive. Different probing radiations allow to access different structural and dynamical parameters on different lengthscales, spanning from the size of particles (10 -1000 nm) to the very local internal structure (0.1-1 nm). Moreover, experiments can be designed to enhance the visibility of selected regions of the aggregates without significant chemical drawbacks. The combined use of laser light, X-ray, and neutrons techniques will be presented as a powerful tool to probe the structural properties of different systems, polymeric nanofibrous or porous scaffolds, nanoparticles, nanoemulsions, also in interaction with mucus models. The correlation between structural and biopharmaceutical properties appears to be a pivotal point for the development of novel platforms suitable for biomedical application and for the delivery of pharmaceutical compounds also via different administration routes, ocular, intranasal, and inhalation.

Why using microfluidics for nanoparticle synthesis offers you so many advantages

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ABSTRACT

The Nanoparticles are a proven delivery method for nanomedicine (vaccines, drugs, gene therapies), as they offer a range of key benefits over traditional approaches. For instance, a significant part of the medical industry's research and development focuses on Liposomes and Lipid Nanoparticles (LNPs) because they allow enhanced efficacy and efficiency of APIs, bio-targeting, stability and controlled release.

However, there is still a key challenge surrounding some of the traditional methodology used. How to ensure that each and every produced particle is consistent in terms of composition, size and payload?

Microfluidics for nanoparticle synthesis offers so many advantages, it enables control, consistency and precision in the created nanoparticles: excellent monodispersity, consistent (targeted) particle size, high encapsulation efficiency, low sample volume, very low waste, high particle integrity, high reproducibility, high-throughput, linear scalable manufacturing, consistent process/API loading, quick and readily optimizable protocols, preserved particle integrity, encapsulation of hydrophobic or hydrophilic cargo.

Harnessing Nature's Secrets: Microalgal-Derived Extracellular Vesicles as Bio-Based delivery system for next-level Pharmaceutical and Cosmetic applications

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ABSTRACT

Extracellular vesicles (EVs) have emerged as one of the most promising bio-nanovehicles for the delivery of endogenous and exogenous bioactive compounds. To overcome the current challenges faced by EV-based drug delivery approaches, our research group has developed a disruptive platform for the sustainable, renewable and cost-effective production, engineering and quality control of a new type of EVs derived from microalgae, which we refer to as nanoalgosomes. In our previous research, we extensively characterized the bio-chemical and bio-physical properties of nanoalgosomes[1-5]. Additionally, we demonstrated that nanoalgosomes exhibit non-cytotoxic behavior and possess the ability to be up-taken by cellular membranes in various human cell lines and *in vivo*, in *Caenorhabditis elegans* models[6]. Here, we present the inherent bioactivity of algosomes *in vitro*, as well as their biocompatibility and biodistribution in a mouse model, along with their exogenous loading capabilities with different molecules. We first evaluated the intrinsic features of nanoalgosomes, such as their antioxidant and anti-inflammatory bioactivity. Furthermore, we conducted toxicity and biodistribution analyses of nanoalgosomes *in vivo* using BALB/C and athymic nude mice, respectively.

Moreover, we successfully achieved the engineering of nanoalgosomes through membrane labeling and loading with chemotherapeutic drugs (*e.g.*, doxorubicin) and nucleic acids (*e.g.*, siRNAs), evaluating both loading efficiency and functional activity of the loaded algosomes.

Our findings revealed that algosomes possess antioxidant and anti-inflammatory properties and do not induce allergic-like responses. Biodistribution analysis in mice exhibited a distinctive organ-specific tropism, with reduced localization in the liver after 48 hours. The loading experiments highlight the efficacy of nanoalgosomes as a natural, innovative, and sustainable EV-based delivery system. These results underscore the potential of nanoalgosomes as new tools for cell-free therapy or cosmetic applications.

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Self-nanoemulsifying drug delivery systems (SNEDDS) for ocular delivery of natural compounds with SIRT-1 activator activity

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ABSTRACT

Recent studies demonstrated that Sirtuin-1 (SIRT-1) activating molecules exert protective role in degenerative ocular pathologies. These molecules hardly reach the back of the eye, due to poor solubility in aqueous environments and low bioavailability. These difficulties, combined with stability issues, suggest the need for innovative delivery strategies. Within this context, the application of self-nanoemulsifying drug delivery systems (SNEDDS) for SIRT-1 delivery can represent a promising approach due to their ability to load lipophilic drug, easy large-scale production, and high stability [1, 2].

SNEDDS can be considered an "advanced" formulation compared to micro- and nanoemulsions, since they are emulsified directly in-situ, avoiding drug loss during storage [3]. SNEDDS, is a very simple formulation, consisting of three components in an anhydrous mixture: oil, surfactant and co-surfactant. The choice of the starting materials and the construction of ternary plot is the crucial point to obtain system with suitable properties for ocular delivery [4-6]. The aim of the work was to design and optimize SNEDDS intended for ocular administration for the delivery of different SIRT-1 agonists such as resveratrol (RSV) and melatonin (MEL) for potential diabetic retinopathy treatment.

Pre-formulative studies were performed by a Design of Experiment (DoE) approach to identify the emulsion zone. SNEDDS were then prepared by low energy method. The optimization phase was carried out using Response Surface Methodology (RSM). Four types of SNEDDS consisting of different surfactants (Tween® 80, Tween® 20, Solutol® HS15, Cremophor EL®) were optimized to achieve best parameters for ocular administration. Stability studies revealed the most stable system composed with Tween® 80 that was, therefore, selected for further physico-chemical and technological studies.

Optimized formulation was prepared with Capryol® PGMC, Tween® 80 and Trascutol® P and loaded with RSV or MEL. RSV loaded-SNEDDS and MEL loaded-SNEDDS were reconstituted with simulated tear fluid (1/10 by volume) and subjected to slight agitation prior to characterization studies to simulate blinking phenomenon and mimic their behaviour in ocular environment after administration.

Moreover, SNEDDS were evaluated for several parameters such as: mean size (<50 nm), homogeneity (PDI less 0.2), emulsion time (around 40 seconds), clarity by turbidimetry (about 100% transmittance), drug content (> 90%), mucoadhesion strenght, *in vitro* drug release, pH and osmolarity, stability to dilution and cloud point. Finally, *in vitro* evaluation was performed on rabbit corneal epithelial cell line (SIRC) to assess their potential cellular toxicity.

Further studies are currently ongoing to deeply assess their potential application for ocular drug delivery.

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Saturday October 7, 2023

Oral Presentations

Organs-on-a-Chip: A new told for studying human physiology

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ABSTRACT

Between 60 to 90% of the drugs that successfully pass animal trials fail in human clinical trials. This poor statistic demonstrates the urgent need for a human-relevant model. Micro-engineered cell culture models, termed Organs-on-Chips, have emerged as a new tool to recapitulate human physiology and drug responses. Multiple studies and research programs have shown that Organs-on-Chips can capture the multicellular architectures, vascular-parenchymal tissue interfaces, chemical gradients, mechanical cues, and vascular perfusion of the body. Accordingly, these models can reproduce tissue and organ functionality and mimic human disease states to an extent thus far unattainable with conventional 2D or 3D culture systems. In this talk, we will present two approaches of using this technology. The first, will demonstrate how drug can be tested by linking of 8 human-Organ-on-a-Chip and showing results that are comparable to clinical data. Furthermore, we demonstrate how to exploit the micro-engineering technology in a novel system-level approach to decompose the integrated functions of the neurovascular unit into individual cellular compartments, while retaining their paracellular metabolic coupling.

On-chip simulation of the efficacy and safety of novel drug delivery approaches: achievements and challenges.

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ABSTRACT

Organ-on-chip (OoC) technology aims at developing and validating animal-free methods that are relevant to human health for testing therapies and studying diseases. OoC devices are widely used in laboratory research for assessing efficacy and toxicity of novel therapeutical approaches including advanced delivery strategies. In human tumor treatments, for instance, OoC can be used to recapitulate the dynamic identity of tumor microenvironment (TME) which is a key factor in cancer differentiation, proliferation, invasion, metastasis and therapeutic response. Indeed, tumorigenesis process occurs under a coordinated interplay between oncogene-mediated cell transformation and TME evolution.

Our group has introduced a novel bioengineered inspired strategy to produce viable 3D human tissue-equivalent in which cells dwell and operate within their own native extracellular matrix (ECM). Following this approach, we reconstructed histological competent portions of human tumor that recapitulate the same cell-ECM dynamic reciprocity of their native counterpart. Spatial and temporal tumor heterogeneity arise spontaneously as a consequence of the recapitulation of the native TME allowing to depict important features of tumor progression and invasion. For instance, several cancer hallmarks such as epithelial-mesenchymal transition (EMT), stromal and desmoplastic reaction, aberrant angiogenesis and multi-drug resistance have been all or in part recapitulated. In this lecture, the competence of these models to recapitulate intra- and extra-tumor heterogeneity and to depict the complexity of TME in vitro will be presented and discussed along the possibility of their use to optimize and design personalized therapeutic approaches.

Microfluidic Production of Biomimetic Liposomes for personalized therapy of metastatic melanoma.

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ABSTRACT

Biomimetic hybrid nanoparticles (Nps) have been developed by exploiting the combination of materials such as lipids, polymers, and metals, with cellular components to produce novel drug nano-delivery systems with an intrinsic capacity to be ignored by everything except their target. Several studies have shown biomimetic Nps potentiality useful for cancer treatment, exploiting their ability to evade the immune system and immunological reactions, improve the stability and longevity of the NPs themselves, bypass biological barriers, prevent non-specific uptake by healthy cells, and target the desired cells affected by the pathology [1].

In this study, we explored the application of the microfluidic technique to the production of biomimetic liposomes through direct fusion between synthetic lipids and the cell membranes (CM) extracted from a metastatic melanoma cell line (MM) derived from a patient biopsy specimen (hybrid liposomes). Here, to address the difficulty to break the CM by purely hydrodynamic forces in microchannels, the coupling of external force fields to microfluidic reaction channel is suggested and a microfluidic sonication strategy for one-step and continuous generation of liposomes and hybrid liposomes is proposed (Fig. 1). Two polypropylene microfluidic devices, fabricated using 3D printing technology with different geometries were tested. The intricate internal architecture in both geometries, enabled a passive adequate degree of mixing of the two phases containing lipids and CM, respectively, thus producing high-quality monodisperse hybrid liposomes [2,3].

To evaluate the best hybridization conditions, we produced three hybrid liposome formulations starting with 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 bare 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.

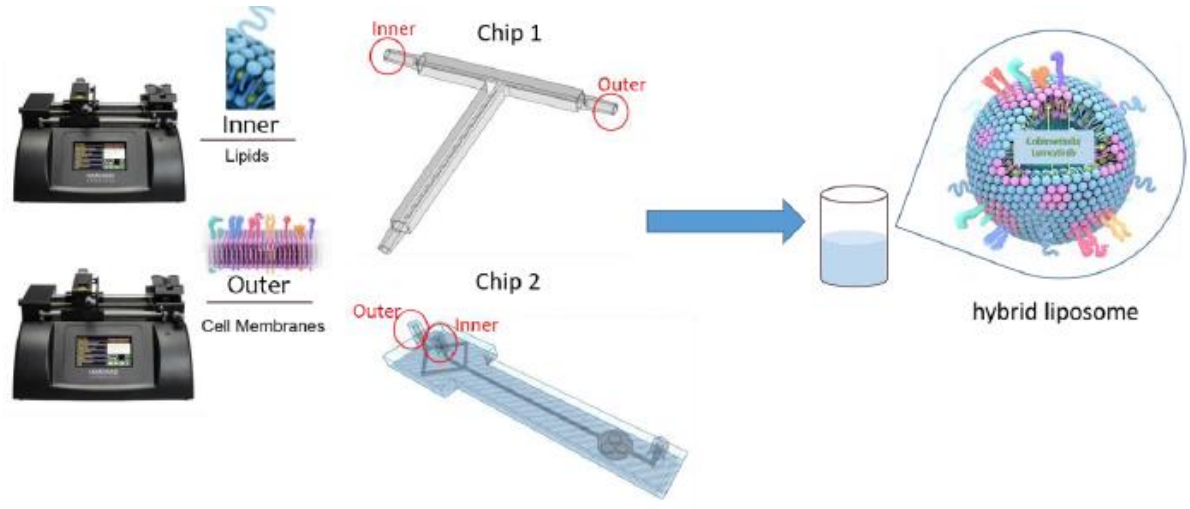

Fig. 1. Schematic representation of set up producing hybrid liposome through microfluidics.

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Nano-in-nanofibers advanced dressing for spatio-temporal delivery of growth factors (GFs) in chronic wound healing

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ABSTRACT

The delivery of GFs to a wound site has the potential to shorten the healing time for chronic wounds and eliminate or significantly reduce scar formation after healing. However, the direct application of GFs as intralesional injection or topical application faces various technological and biological challenges that strongly limit its clinical relevance. Integrating GFs into 3D scaffolds represents an innovative approach that can overcome the fundamental problem of achieving the correct spatiotemporal delivery of GFs to mimic their physiological performance *in vivo*. This therapeutic approach makes possible a controlled delivery of GFs in the proximity of the wounded area (thus avoiding side effects and exposure of non-target sites) and using a combination of different encapsulation techniques is possible to design advanced dressings with varying levels of complexity to provide a fine-tuning of the local drug delivery to the chronic wound site.

The overall objective of this work is to prepare a multifunctional dressing for the correct spatiotemporal delivery of GFs for diabetic foot ulcer (DFU) patients. This dressing is based on a polymeric fiber obtained by electrospinning incorporating endothelial growth factor (EGF)-loaded nanoparticles to achieve a nano-in-nano delivery system. Polymeric nanofibers with a core-shell structure and an average diameter of 309 ± 64 nm were obtained with coaxial electrospinning using poly (vinyl alcohol) (PVA), polyvinylpyrrolidone (PVP), and hyaluronic acid (HA). PVA (core)/PVP-HA (shell) nanofibers were defect-free and had a uniform thickness, as confirmed by SEM and TEM microscopy. PLGA:Poloxamer nanoparticles (NPs) were prepared by a modified solvent diffusion technique, encapsulating the GF in the presence of human serum albumin (HSA) and heparin sodium salt (Hp) as stabilizers. The developed nanocarriers were first characterized for morphology, size, encapsulation efficiency, and release kinetics *in vitro,* then loaded into the nanofiber shell. The homogeneous dispersion of NPs was confirmed by TEM and confocal laser scanning microscopy. The nano-in-nano wound dressing was tested for functional characteristics, including mechanical strength, porous microstructure, stability, hydration, and polymorphic/amorphous transitions. Preliminary release

experiments on a model protein in simulated wound fluid showed sustained release for over 7 days, a period suitable for a wound regeneration effect.

In conclusion, PLGA:Poloxamer NPs were successfully loaded into a core-shell nanofiber to attain local delivery at a sustained rate of GFs. The composite dressing developed here is promising to handle DFUs, and further studies are in progress to evaluate its in vitro wound healing activity.

Thursday October 5

Poster Session I

Drug-free hybrid nano-architectures modulate the metastatic behavior of pancreatic ductal adenocarcinoma in alternative *in vivo* models

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ABSTRACT

Metastasis is defined as the spreading of cancer cells in the body, and it accounts for most deaths and relapses in oncological patients. The spreading of cancer cells is associated to the epithelial-to-mesenchymal transition (EMT) phenomenon, which confers mesenchymal, hence migratory, properties while suppressing the epithelial features of cells.1 Noble metal nanoparticles, as gold and copper nanoparticles, are appealing to shift the paradigm in clinical oncology but very little is known concerning their antimetastatic behaviors. Noteworthy, the main hurdle for inorganic nanoparticle translation to clinics is their persistence in the body, which can be overcome with a safety-by-design approach to produce ultrasmall-in-nano architectures (NAs).2

Non-toxic concentrations of gold- and copper-containing NAs were tested on 2D cultures of pancreatic ductal adenocarcinoma cell line, and their time-dependent effects on the expression of EMT-related genes were evaluated using real-time PCR. The *in vivo* effect of NAs on tumor size and metastasis spreading was monitored for three days after a single application on optimized chorioallantoic membrane (CAM) model. Gene and protein expressions of EMT-related factors were explored in harvested tumors, and *Alu* sequence quantification was employed to follow the migration of cancer cells to the lower CAM. In *in ovo* models of pancreatic cancer, gold- and copper-containing NAs slowed down the progression of metastasis in upper and lower CAM, with a concomitant alteration of gene and protein expression of EMT-related factors up to 72h. Remarkably, the importance of alternative *in vivo* models as CAM was stressed by the different activity exhibited by NAs shifting from 2D cultures to CAM models. In conclusion, NAs modulation of tumor growth and metastasis spreading in *in ovo*

models, besides their efficient excretion from the body, is a promising starting point for the formulation of novel approaches for the treatment of pancreatic cancer metastasis.

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Verteporfin-loaded nanoparticles targeting cMET: a novel approach to tackle desmoplastic tumors

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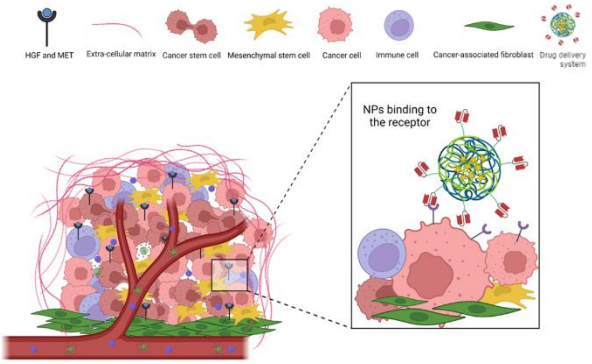
ABSTRACT

In 2023 lung cancer will be the leading cause of cancer death [1]. Commonly, lung cancers develop desmoplasia, a condition defined by the formation of excessive ECM, which can block the drugs' ability to reach the tumor interstitium. In cancer cells, the activation of the mechanotransducers YAP and TAZ regulates the properties of the ECM. The multitarget drug Verteporfin (VP), as a result of its ability to disrupt the YAP-TEAD interaction, can block the secretion of ECM components and the recruitment of pro-tumorigenic cells, thus arresting cancer progression and reducing ECM stiffness [2].

We developed a nanoplatform loaded with VP and covalently bound to a newly-synthetized scFv specific for the mesenchymal-epithelial transition receptor (MET), which is overexpressed on the cancer cellular membrane. The NPs are capable of exploiting MET as anchor of attachment to enter the cells.



We synthetized a functional polymer, Mal-PEG-NHCO-PLGA, starting from PLGA (66 kDa) and PEG (5 KDa). The maleimide functionalities in the polymer are located at the terminal end of the PEG; therefore, upon formulation of NPs, the hydrophilic PEG should facilitate the presentation of maleimide functionalities, making them available for covalent binding with the cysteine groups of the scFv. Mal-PEG-NHCO-PLGA was characterized by GPC to assess the molecular weight and 1H-NMR to confirm the structure. NPs composed of 25% wt PLGA (48kDa, structural polymer), 25% wt PLGA-PEG (90kDa, stealth polymer) and 50% wt Mal-PEG-NHCO-PLGA were prepared by the microfluidic platform NanoAssemblr® Benchtop. They showed homogeneous size of 75 ± 7 nm (PDI 0.12 ± 0.05) and negative surface charge (-21mV). After bioconjugation, performed in pH 7.4 PBS (0.5% wt Tween 20) at RT for 3.5 h, the NPs reached size of 195 ± 50 nm (PDI 0.31 ± 0.11) and neutral surface charge (-0.3 mV); moreover, the TEM analysis reveals NPs with spherical shapes. Bioconjugation efficacy was evaluated by BCA assay and it was 58 ± 17 % (~ 93 µg of ScFv/batch). NPs were loaded with VP, the drug loading was evaluated by spectrofluorometer method and it was 12

ug of VP/mg of NPs. The release profile of VP showed a triphasic pattern: an initial burst release of around the 35%, over the range of 4–16h a released amount of 64% was achieved and finally the release was accomplished at 96 hours. Western-blot analysis revealed that NPs conjugated with the ScFv can specifically bind MET, while there is no unspecific interaction between MET and NPs without the scFv.

NPs internalization was qualitative assessed on lung carcinoma cells (A549) by confocal microscopy. Results displayed NPs massively enter the cells after 1.5 h of incubation.

In conclusion, we developed a versatile polymeric platform able to be functionalized with specific ScFv and efficiently deliver drugs to MET expressing cells.

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Cell membrane-derived nanoparticles for active targeting to tumor cells

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ABSTRACT

Cell membrane-deriving nanosystems may display special features when employed in drug delivery, such as specific cell recognition, long blood circulation, and reticuloendothelial system escaping, due to retained membrane structure and composition [1, 2].

We have developed a hybrid nanosystem composed of colorectal cancer cell membranes fused with biocompatible synthetic lipids. A previously described method was modified to isolate and improve the purification of cell membranes fragments [3], and the presence of residual genetic material was studied, to rule out any possibility of retaining mutagenic components in the membrane fraction. Hybridization with synthetic lipids was achieved by exploiting two different techniques: extrusion through decreasing pore size polycarbonate membranes (ex-hybr) or using a microfluidic device (μQ-hybr). The obtained formulations were compared in terms of chemical-physical properties, stability, process yield and membrane hybridization efficiency. The *in vitro* internalization of μQ-hybr, identified as the most promising formulation, was studied by confocal microscopy and flow cytometry. This hybrid nanosystem showed good cellular uptake already after 30 minute incubation in three different cell lines, with a preferential internalization in colorectal cancer cells (parent cell line).

The obtained preliminary data confirmed that a pure and stable bio-inspired nanosystem was set up with good production yield; moreover, thanks to the cell-derived membrane components, its cellular uptake sensibly increased in comparison to artificial liposomes with the same lipidic composition, reaching the highest value in the homotypic internalization into the parent cells.

These promising results suggest that nanosystem functionalization using cell membranes might be an efficient approach for a unique cancer targeting nanovehicle to be employed for drug delivery applications. In future studies, a protein profile characterization will be performed on isolated membranes and hybrid systems to study the retention of macromolecules involved in target cell invasion process. Moreover, *in vivo* studies on murine colorectal cancer models will be performed to further investigate the biodistribution, safety and tissue accumulation of this innovative hybrid nanosystem.

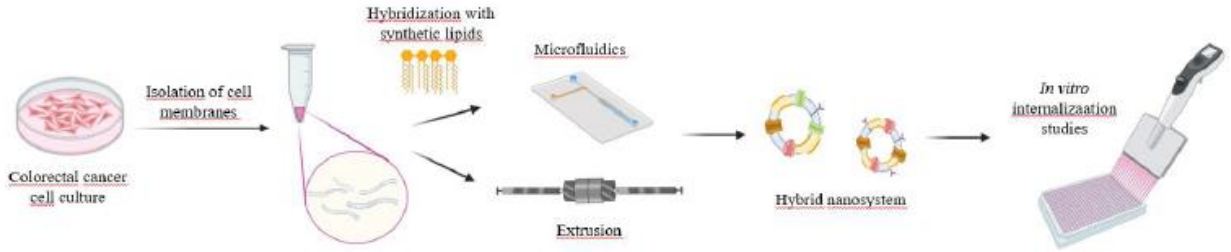

Fig. 1: Schematic representation of the study.

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NIR-light responsive AuNRs/polyurethane based-electrospun membrane for synergistic chemo-photothermal therapy

Annalisa Martorana[1*]; Giorgia Puleo[2,3]; Giovanni Carlo Miceli[1]; Mariano Licciardi[1], Calogero Fiorica[1], Giovanna Pitarresi[1]; Fabio Salvatore Palumbo[1] and Gaetano Giammona[1]

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ABSTRACT

Post-surgical loco-regional implantation of smart biomaterials, to prolong and control the release of chemotherapics in response to external or micro environmental specific stimuli, is a promising strategy in order to retard tumour recurrence. In such localized approach, hyperthermia can be integrated with chemotherapy creating a synergistic effect due to the increasing drug release and/or to the temperature effects. When exposed to high temperatures (up to 45 °C), cancer cells undergo apoptosis, for this reason the combination of hyperthermia-chemotherapy may reduce the required dosage of anticancer drugs and improve the overall therapy effects. Polyurethane nanofibrillar electrospun membranes, due to their high surface-volume ratio and to their favourable mechanical properties, can represent optimal platform for loco-regional smart delivery. Electrospinning procedure offers the opportune versatility in order to load high amounts of chemotherapic together with photothermal (PTT) agents [1].

In this work, a redox-responsive biodegradable polyurethane urea (PUU) [2] was chosen to produce paclitaxel (PTX) and gold nanorods (AuNRs) loaded electrospun membranes for combined near-infrared (NIR) light responsive release and hyperthermic cytoreductive effect (Fig.1). Three different AuNRs concentrations were tested and electrospinning conditions were optimized to fabricate AuNRs loaded membranes. The obtained nanocomposite electrospun membranes showed sustained release of PTX (about 7% released in 10 days), while AuNRs served as PTT agents, which exhibit an increase of temperature reaching 45 °C when exposed to NIR light (810 nm) for 4 minutes (3 W/cm^2) with the higher concentration of AuNRs selected (Au_1550µg).

The PUU/AuNRs/PTX electrospun membranes showed an excellent PTT effect and a release triggered by NIR light irradiation, leading to a significant cytotoxic effect in two tested cancer cell lines: human breast cancer (MCF-7) and human colon cancer (HCT-116) cell lines.

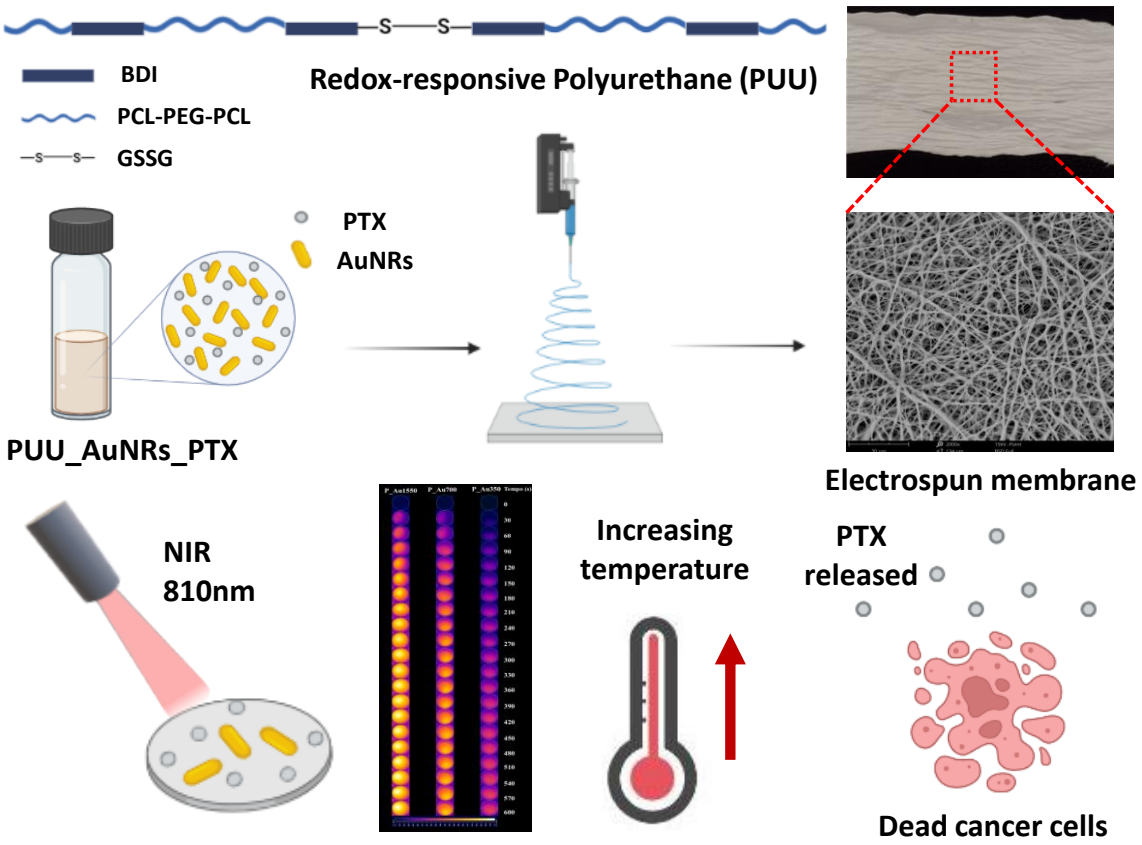

Fig. 1. Schematic illustration of PUU_AuNRs_PTX processes.

This system could potentially allow the release of paclitaxel in a controllable manner near the tumor area due to the PTT effect. The results showed an enhancement cytotoxicity when combining drug with PTT effect, highlighting the huge potential of this drug delivery system as a promising approach for future anticancer treatments.

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Stealth cationic liposomes for plasmid DNA gene therapy

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ABSTRACT

Gene therapy has emerged as a remarkable therapeutic option with immense potential for various biomedical applications in the future. The success of gene therapy largely depends on the development of efficient delivery technologies. Negatively charged genetic material faces challenges in crossing cell membranes and is prone 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, employing cationic lipids (52 mol%) with a permanent positive charge (DOTAP) or ionizable (DLin-KC2-DMA). These lipids allowed for complexation and encapsulation of plasmid DNA. The formulations also included cholesterol (38.5 mol%) for stability and HSPC (8 mol%) as a helper lipid. Additionally, PEG-DSPE (1.5 mol%) 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 direct complexation during liposome formation. The liposomes were characterized using dynamic light scattering (DLS) for size, PDI, and zeta potential. 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 ranged from +20 to +30 mV for DOTAP liposomes, while DLin-KC2-DMA vesicles had a zeta potential of -10 mV. 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, the liposomes were tested on myogenic cells to evaluate pDNA transfection. 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.

Thiol reactive PEG linkers for high loading ADCs

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ABSTRACT

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, inducing aggregation 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 polyethylene-glycol (PEG) based linkers 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 of a linker containing a ValCit dipeptide for 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 two PEG 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 synthesized ADCs appeared to be highly homogeneous when analyzed by native HIC analysis, eluting as a major peak corresponding to the fully conjugated species (DAR8). The same analysis well confirmed that increasing the PEG length promotes a reduced hydrophobicity of the entire conjugate. Remarkably, when thermally stressed at 40°C and 60% of humidity over 4 weeks, the aggregates content decreases as the PEG length increases. This stability study in solution highlighted that all PEG linkers allow to double the amount of drug while maintaining the same level of aggregation of a non-PEGylated DAR4 reference ADC. When tested for the *in vitro*, all ADCsexhibited a IC50 in the nanomolar range against HER2+ cell lines (SK-OV-3 and SK-BR3), which suggests that the PEG length does not interfere with the release of the free MMAE by lysosomal enzymes. Pharmacokinetic studies in BALB/c mice suggested a clear relationship between PEG length and clearance *in vivo*. While the half-life of the ADCs bearing PEG12 and PEG8 is very close to that of the parental antibody, the same is reduced in the case of the ADC based on PEG4. *In vivo* studies again

demonstrated an inverse correlation between apparent hydrophobicity and antitumor activity. In fact, in a SK-OV-3 mouse xenograft model, the highest antitumor activity was observed for ADCs bearing PEG chains sufficiently long to maximize the *in vivo* exposure (PEG8 and PEG12).

References:

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Formulation & scale-up of delamanid nanoparticles *via* emulsification for oral tuberculosis treatment

Nicholas Caggiano[1*], Madeleine Armstrong[1], Joanna Georgiou[1], Aditya Rawal[2], Brian Wilson[1], Rodney Priestley[1], Robert Prud'homme[1]

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ABSTRACT

Delamanid is a small molecule anti-tuberculosis therapeutic used to treat drug-resistant tuberculosis (DR-TB), a strain of the disease associated with higher mortality rates and the need for harsher therapies. However, the bioavailability of delamanid is limited by its hydrophobicity and crystallinity. Nanoparticle encapsulation is an attractive route to increase the bioavailability of delamanid by increasing the specific surface area available for drug dissolution and by forming an amorphous drug core. Initial investigation of nanoprecipitation as a formulation route revealed significant incompatibility between the solid drug core and commonly employed nanoparticle stabilizers due to trifluoromethyl and nitro groups present on delamanid. To address this, emulsification was investigated as a route to form stable drug-loaded nanoparticles while avoiding the in-situ formation of a solid drug core, as the delamanid remained solubilized in a liquid core during emulsification. Inexpensive, naturally derived emulsifiers, including lecithin and functionalized cellulosics, were employed as stabilizers. Depending on the stabilizer, emulsification produced stable particles 100-600 nm in diameter, which were spray dried to produce a dried powder. A 1:1 mass ratio of lecithin and HPMC was found to be optimal in producing smaller particles (250 nm) which were also robust enough for spray drying. An in vitro dissolution assay revealed that the dried emulsion formulations produced enhanced dissolution kinetics compared to bulk crystalline delamanid, and the powders remained stable against aging-induced crystallization at elevated temperature and humidity. Solid-state NMR measurements revealed that the spray-dried emulsions were semicrystalline but more robust to temperature and humidity induced crystallization than a comparable amorphous solid dispersion.

Properties tuning of an *in situ* polymeric powder for wound healing by the use of cosolvent or functional excipients

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ABSTRACT

Outline of the project

Nowadays, many products for the treatment of acute and chronic wounds are available on the market [1]. Among them, hydrogels based on polysaccharides such as alginate and chitosan are promising wound care materials for skin regeneration due to their ability to hold high content of water maintaining the moisture of the wound, their conformability and the capacity to release encapsulated drugs [2]. Moreover, when properly mixed, natural polymers can produce formulations able to exert better properties compared to the starting materials [3]. The aim of this work has been to develop a spray-dried powder composed of alginate, pectin, and chitosan able to become *in situ* gel for wound healing applications. Moreover, to enhance *in situ* gelling ability and tune gel properties, the use of cosolvents or functional excipients has been investigated.

Experimental section

Alginate/pectin/chitosan (APC) polymeric blends were produced through mini spray drying setting total polymers concentration at 0.15% (w/v). Concerning the powders produced with a cosolvent, feed solutions with 5, 10, and 20% V/V of ethanol, 20% V/V of ethanol/acetone (1:1), and 5% V/V of isopropanol were tested. Whereas, sodium bicarbonate and carbonate ammonium were selected in the range from 10 to 2.5 % w/w. The influence of cosolvent or functional excipient was investigated in terms of the particle size distribution and morphology as well as fluid uptake ability. Moreover, FTIR spectra, viscoelastic properties, and water vapor transmission rate were evaluated.

Results

All powders have the ability to gelify very quickly when in contact with wound exudate, especially formulations with 5% of ethanol (APC 117-5Et) and bicarbonate (APC 117-10Bic) that showed very fast gelation (less than 5 mins and 30 sec, respectively). The reason for this can be explained by the morphological and physical-chemical properties of these particulate powders. In fact, FIB-SEM showed submicrometric assembled powders when cosolvent was used, while hollow particles when bicarbonate was used as an excipient, allowing an increase in the contact area with wound fluid (Fig.1). Moreover, the rheology analysis allowed highlighting the difference in gelation behavior due to the presence of cosolvent or functional excipients.

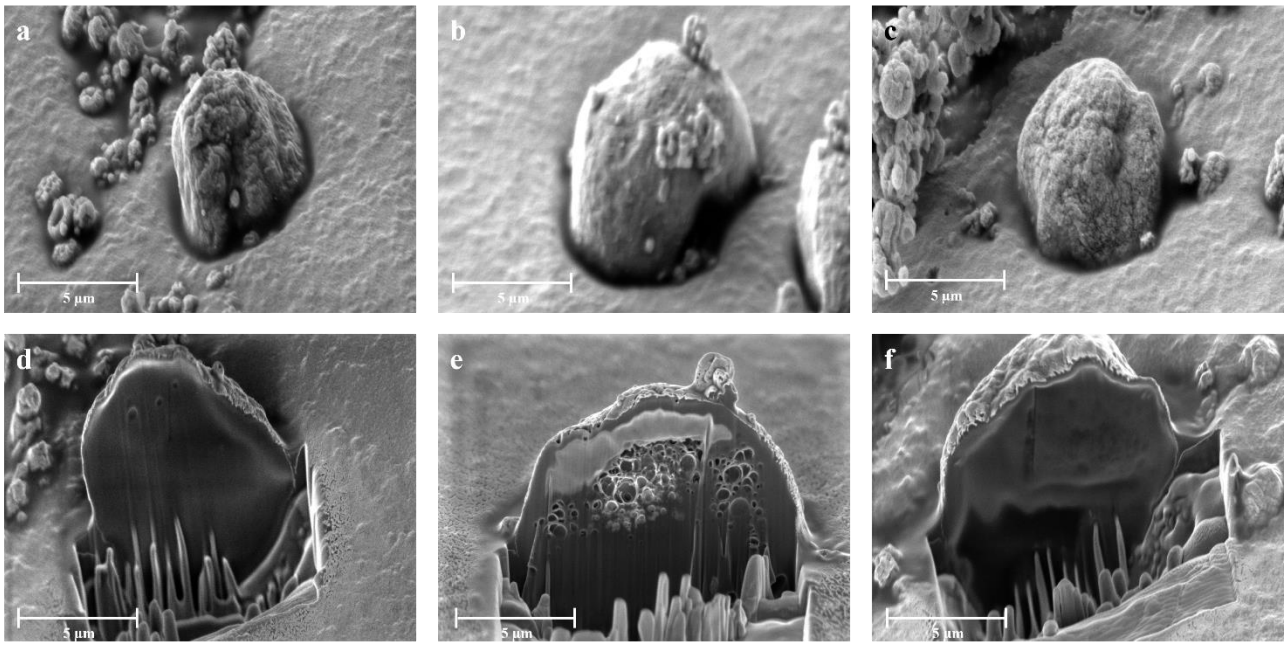

Figure 1. External morphology of APC 117 (a), APC 117-5Et (b), APC 117-10Bic (c) obtained by SEM microscopy and cross-section of APC 117 (d), APC 117-5Et (e), APC 117-10Bic (f) obtained by FIB-SEM microscopy.

Conclusions

Spray drying was successfully used to generate alginate/pectin/chitosan powders capable to move rapidly from powder to gel when in contact with wound exudate. The possibility to use cosolvent (ethanol) as well as the addition of functional excipients (sodium bicarbonate) was verified demonstrating the huge versatility of this polymeric blend to incorporate other solvents and excipients.

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Design and development of local treatments for the post-surgical microenvironment of glioblastoma

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ABSTRACT

Glioblastoma (GBM) is an incurable primary brain tumour. Most GBM patients undergo surgery prior to chemoradiation, but recurrences inevitably lead to patient death. The brain physiological regenerative responses following tumor debulking have a beneficial role in the healing process. However, they also evoke characteristic time-dependent peritumoral immune responses which can promote the formation of recurrences[1]. We have previously developed a nanomedicine-based hydrogel to be injected in the post-surgical cavity of GBM to deliver dual-drugs around the resection cavity borders at therapeutic concentration[2]. These systems were able to delay the onset of recurrences but not to inhibit their onset[3]. The crosstalk between glial, immune and GBM cells at the surgical borders and their impact on recurrences still lacks appropriate characterization and this will be crutial to select drug combinations able to stop recurrences[4]. Here, we aim at dissecting the GBM post-surgical microenvironment (SMe) over time and space to identify therapeutic targets and develop tailored GBM therapies to avoid the onset of recurrences.

We developed a tumor resection model in transgenic mice bearing GL261 tumors and established a chronic intracranial window post-surgery by biphotonic imaging. Nuclear imaging was performed at defined time points using 18F-FDG, 68Ga-RGD and 99mTc-DTPA to evaluate tumor metabolism, neoangiogenesis, infiltrating tumor cells metabolism and BBB permeability. The dynamics of the inflammatory landscape following surgery was characterized by blood sampling and post-mortem analysis on brain samples (peripheral and local immunophenotyping by multiparametric flow cytometry; brain clearing and ultramicroscopy). We analyzed the dynamics of recruitment and localization of immune cells coming to the resection site from the brain parenchyma or from the periphery from surgery to recurrence. BBB disruption was observed post-surgery followed by a recovery within three days. Based on the results obtained we finally tested two therapeutic approaches combining the local administration of cytotoxic agents (Gemcitabine or Doxorubicin prodrugs) loaded

in lipid nanocapsules hydrogels with the repeated systemic administration of immunomodulatory or anti-inflammatory drugs showing an increase in survival of GBM-bearing mice.

This comprehensive study expands the knowledge on the SMe by analyzing the impact of BBB disruption on immune cells recruitment and the role of lymphoid and myeloid populations - as well as macrophages and microglia - on the onset of tumor recurrences. Moreover, it proposes rationally designed combinatory treatments able to target the identified cellular targets to delay or inhibit the onset of recurrences.

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Preliminary *in vitro* and *in vivo* studies of (S)-(–)-MRJF22 loaded-NLC for potential treatment of uveal melanoma

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ABSTRACT

Uveal melanoma is one of the most diffused ocular cancers, and, it is mainly located in the choroid (90%), but also in the ciliary body (7%) and in the iris (3%) [1]. Mainly due to early appearance of liver metastasis, it causes mortality of 80% in the first year and 92% in the second year [2]. Until today, treatment approaches are surgery, radiation, and enucleation. However, due to the involvement of HDAC and σ receptors, a promising approach could be the administration of (*S*)-(–)-MRJF22, a new-synthetized prodrug of (±)-haloperidol metabolite II conjugated with valproic acid, for its activity on both targets [3]. Considering the drawbacks of ophthalmic administration, a successful approach for ocular targeting is drug encapsulation into drug delivery systems [4]. Basing on these considerations, (*S*)-(–)-MRJF22 was encapsulated into a nanostructured lipid carriers (NLC) platform, which was subsequently functionalized with PEG, namely (*S*)-NLC and (*S*)-PNLC. The produced nanosystems demonstrated particle size adequate for ocular administration (< 200 nm). Since zeta potential values were found to be neutral, stability studies were carried on, storing the samples for 30 days in Turbiscan® Aging Station, at 25°C, 36.5°C and 50°C, resulting to be stable. Moreover, nanoparticles morphology was investigated using transmission electron microscope. The release of the prodrug from the nanoparticles, assessed by *in vitro* in Franz diffusion cells, demonstrated a similar behavior for both formulations, with a slow and prolonged prodrug release. Human corneal epithelium cells (HCE-2) were used to assess the cytocompatibility of the samples, as well as the free prodrug. Furthermore, Draize ocular irritation test was performed on New Zealand albino rabbits, confirming the safety of both the

nanosystems. In conclusion, the developed NLC loaded with (*S*)-(–)-MRJF22 demonstrated to be a promising strategy for the treatment of uveal melanoma. *In vivo* biodistribution studies are still ongoing, in order to verify the targeting ability of the nanoparticles and their ability to reach the posterior segment of the eye.

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Preclinical development of a microMESH implant for the combinatorial delivery of nucleic acid-based nanomedicine and chemotherapeutics in the treatment of brain tumour

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ABSTRACT

Gene therapy has been proposed for a variety of inherited and acquired human diseases[1,2]. Despite the progress in the field, the delivery of nucleic acids (NA) to target cells and tissues is still challenging: the use of NA is mostly limited by their instability in biological environment and inability to cross cell membranes to reach specific intracellular compartments. Moreover, their application in brain diseases is even more hampered by the presence of the blood brain barrier (BBB) that limit dramatically the accumulation of drugs and nanomedicines within the brain.

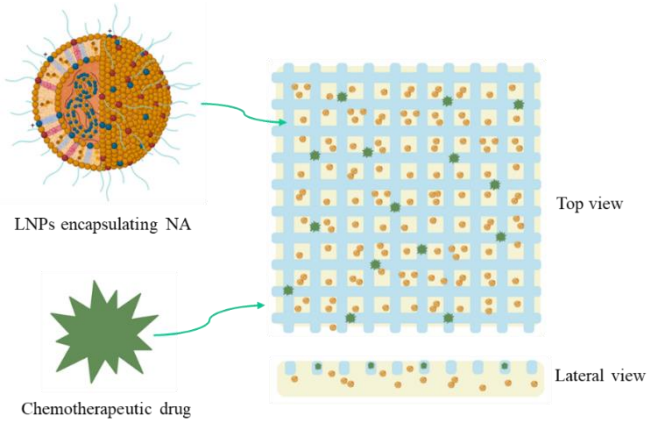

Figure 2. Representation of microMESH loaded with LNPs in the PVA layer (yellow) and chemotherapeutic drug in PLGA strands (light blue)

Here, lipid NPs encapsulating pDNA for reducing drug resistance have been developed and included in a novel dual-compartmental polymeric implant - microMESH to enable the local therapy of brain tumor. First, the lipids composition of LNPs, based on ionizable lipids, and the correct N/P ratio have been fully characterized to understand their effect on the nanoparticle physical-chemical properties and optimize the encapsulation of the anionic cargo and the transfection properties of the particles.

Polymeric microMESH, was fabricated following a top-down process previously described by the authors[3]. microMESH has been extensively characterized for its geometrical and physico-chemical

properties via atomic, optical and electron microscopy. The precise and regular geometry provides to the device flexibility and its ability to intimately interact with cells. Moreover, microMESH supports the sustained delivery of the nanomedicines deep into the tumour tissue circumventing the impermeable BBB.

Under physiological conditions (PBS buffer), the loading and release profile of the therapeutic agents from microMESH have been studied showing an immediate release for hydrophilic molecules (over a few days), loaded into a PVA microlayer, and a prolonged one for hydrophobic drugs (sustained for months), loaded in the PLGA micronetwork. The biodegradation of microMESH under physiologically relevant conditions was assessed qualitatively via optical imaging and quantitatively via q-NMR and chromatographic analyses. Nanoparticles-loaded microMESH showed a 60% EE% and a 98% of release from the PVA layer in 1h. The dimension and morphology of LNPs after the release from the PVA layer were assessed by DLS and TEM respectively, showing to be stable; in particular, a polymeric corona seemed to be formed on LNPs surface which caused an increase in dimension that reduced over time with the dissolution of the corona. Then, the microMESH was loaded with chemotherapeutic drug molecules dispersed within the PLGA micronetwork. The polymeric device was thus evaluated for a combinatorial release of LNPs encapsulating NA and drugs in glioblastoma cell lines. The combination of NA-loaded LNPs and microMESH technology are expected to boost patient-specific brain tumor therapy by a combinatorial release of drugs with a synergic action.

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Injectable thermosensitive hydrogel for local and controlled delivery of siRNA polyplexes to ovarian cancer ascites

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ABSTRACT

In the oncology field, small interfering RNA (siRNA) based therapy is not that far advanced yet in clinical reality due to siRNA *in vivo* instability and low cell transfection efficiencies of naked siRNA. To overcome these challenges, one of the strategies used is the complexation of anionic siRNA via electrostatic interactions with cationic polymers, leading to the formation of polyplexes [1].

For this, methoxypoly(ethylene glycol) poly[2-(dimethylamino)ethyl methacrylate] diblock copolymer was used as polymeric carrier for siRNA condensation. Small polyplexes were tracked *in vitro* using 3D multicellular tumor models (spheroids) [2]. Homo and heterospheroids of avascular ovarian nodule models were optimized by coculturing ovarian cancer cells with primary mouse embryonic fibroblasts in different ratios (2:5, 1:1 and 5:2) mimicking stroma-like conditions. For *in vivo* studies, siRNA polyplexes were loaded into a thermosensitive hydrogel composed of pNIPAM-PEG-pNIPAM (NPN) polymer and injected in a mouse disease model. First outcomes showed how condensed siRNA polyplexes prepared at N/P charge ratio of 5, had a size of 25 ± 2 nm in HEPES solution. For *in vitro* experiments, homo and hetero spheroids with a reproducible size of ~200 μm were developed. Penetration studies revealed that, with increasing the fibroblasts content, the siRNA polyplexes penetration decreased, as visualized in Fig. 1a-b. Importantly, even although the penetration of siRNA polyplexes is strongly affected by extracellular matrix produced by the fibroblasts, the nanoparticles can still reach the core of the ovarian nodulus with a penetration depth of ~60-70 μm. Preliminary *in vivo* studies of siRNA polyplexes loaded into injectable hydrogel revealed that particles were able to reach and penetrate into the tumor nodules localized in the peritoneal cavity of the mouse (Fig. 1c).

To conclude, the NPN hydrogel releasing size-tuned siRNA polyplexes (for at least 7 days) have high potential for therapeutic purposes to exploit penetration into tissues and enhance eradication of the ovarian tumor nodules.

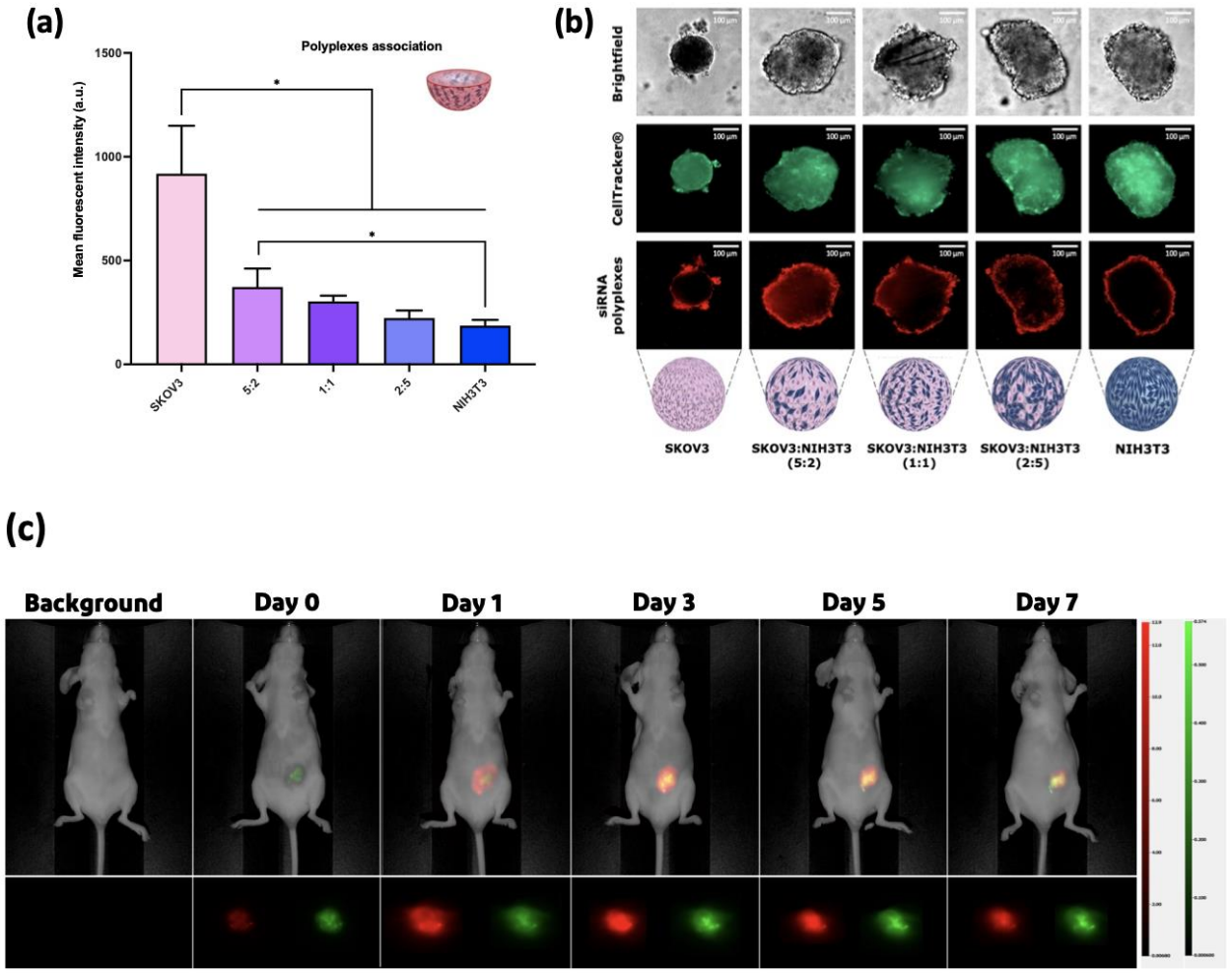

Figure 1. (a-b) Penetration of siRNA polyplexes into homo and heterospheroids. (c) *In vivo* siRNA polyplexes-loaded hydrogel stability monitored for 7 days after injection in the peritoneal cavity (red = siRNA-Cy5.5 and green = PD-Cy7).

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Injectable thermosensitive hydrogel for cardiac delivery of therapeutics

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ABSTRACT

Many genes and pathways have been indicated to play essential roles during heart regeneration and manipulation of these pathways using mRNAs or small molecules have been shown to be promising therapeutic strategies. For this, a dual delivery system composed of mRNA polyplexes and micelle-containing thermosensitive hydrogel, previously complexed with CHIR99201 (a small hydrophobic GSK3 inhibitor and Wnt agonist), was designed allowing local sustained release of mRNA as well as small molecular weight drugs. In this study, the synthesis of mPEGpDMAEMA (PD) diblock copolymer was optimized via RAFT polymerization and this polymer was used as polymeric carrier for mRNA condensation. Condensed mRNA polyplexes showed an average size of 146±11 nm (N/P charge ratio 10) with a positive zeta potential (10.1 ± 0.3 mV). Subsequently, the loading of mRNA polyplexes into a thermosensitive pNIPAM-PEG-pNIPAM (NPN) hydrogel was evaluated to facilitate local and sustained mRNA release. The NPN triblock copolymer synthesized by ATRP polymerization, was used as a loading carrier for CHIR99021 (CHIR) by forming flower-like micelles that encapsulate the hydrophobic drug via heat-shock procedure. The CHIR-NPN gel was then formulated by increasing the CHIR-NPN polymer content, reaching the final concentration of 25% w/w. After 15 days, NPN placebo hydrogels were fully dissolved while drug-loaded hydrogels exhibited much longer degradation times (up to 54 days). This proves that the presence of CHIR affects the stability of the hydrogel, presumably due to its interaction with the dehydrated pNIPAM blocks. Experimental studies revealed a sustained release of the drug over 54 days, demonstrating release kinetics mainly governed by hydrogel erosion. Placebo and CHIR-loaded gels showed temperature-sensitive behavior with a gel point below 37 °C, proving their injectability and *in situ* gelation upon administration, as previously demonstrated. The final dual delivery system, composed of mRNA polyplexes and CHIR-loaded NPN hydrogel, showed the same mechanical properties as the CHIR-NPN gel and demonstrated injectable features at room temperature. *In vivo* pericardial injection of the dual delivery system in healthy mouse hearts was performed thanks to the thermosensitive properties of the hydrogel. After 4 days of treatment, it was shown that the hydrogel was able to trigger the proliferation of cardiomyocytes (Fig. 1). Encouraged by

these data, follow-up studies are planned to evaluate the ability to induce proliferation of cardiomyocytes in infracted hearts upon administration of mRNA-CHIR-NPN hydrogel.

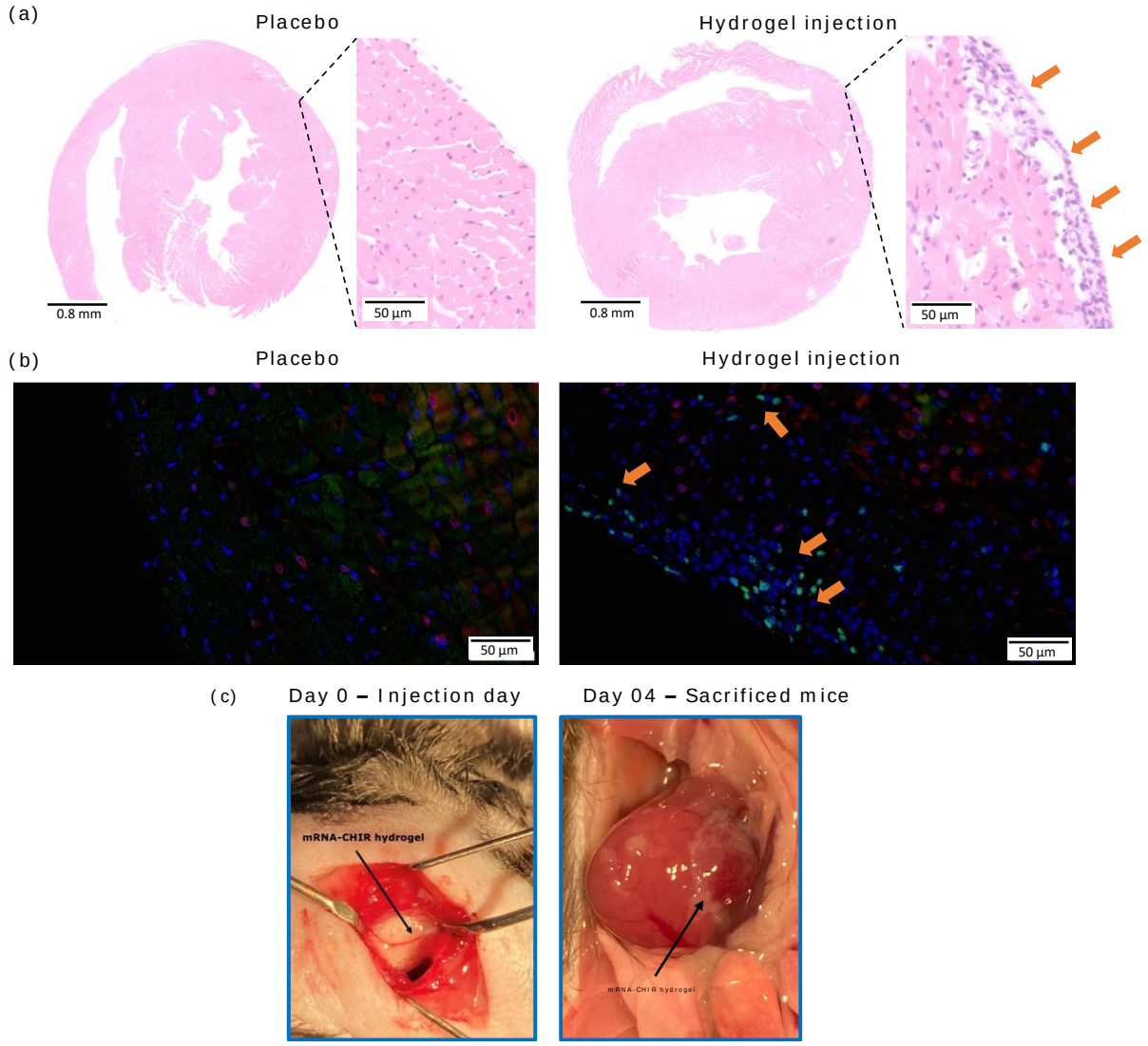

Figure 1. Bioactivity of mRNA polyplexes loaded into CHIR-NPN hydrogel (a) Representative images of H/E staining of mouse heart sections treated with placebo (PBS) or hydrogel 4 days after injection. (b) Immunofluorescence staining 4 days after injection. Hydrogel treatment promotes pericardial cells proliferation compared to placebo group (orange arrow). (c) Optical images of the mouse heart at the day of the injection (left) and at day 4 when the mice were sacrificed (right).

Biopolymer-based strategies toward heavy metal removal and neurodegenerative diseases management

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ABSTRACT

Water bodies deterioration has increased dramatically, mainly due to anthropogenic processes, with heavy metal ions (HMI) being the most persistent and toxic contaminants. These environmental pollutants have been identified as etiological agents for neurodegenerative disorders (Alzheimer and Parkinson diseases) [1]. The design of novel HMI-binding materials based on natural polysaccharides can be a potential strategy to simultaneously apply sorptive matrices for water decontamination and deliver therapeutics for neurodegenerative diseases management.

The system was composed of vinylsulfonated β-cyclodextrin (βCD-VS) and thiol-derivative of hyaluronic acid (HA-SH) exploiting Michael-addition crosslinking reaction (Fig.1a). Structural characterizations of the formulated products were performed under NMR, FT-IR, RAMAN spectroscopy and rheological analyses. To confirm the stability of the system, swelling and degradation studies were carried out with the presence of dithiothreitol as reductive agent. Encapsulation efficiency behavior of the system was studied using hydrophobic (docetaxel, DTX) and hydrophilic (gemcitabine, GCB) model molecules.

As first results, NMR and FT-IR confirmed the structure and degrees of substitution of formulated products. The Michael addition crosslink behind the gelification was proved by rheological measurements and RAMAN spectroscopy. The RAMAN spectrum of the lyophilized gel revealed new peaks around 600-700 cm-1 range that corresponds to -C-S- bond of Michael-addition between HA-SH and βCD-VS groups (Fig. 1b). It was proved that with time under reductive conditions, the HA-βCD hydrogel was stable, while the gel with just HA-SH degraded in 15 minutes (Fig. 1c). Preliminary studies proved that DTX was successfully complexed within the βCD-VS cavity while GCB was entrapped during the gelification process. HA-SH proved to be a good HMI binding material, as also proved before [2].

To conclude, the HA-βCD hydrogels can be exploited as potential platforms for the treatment of neurodegenerative disorders and as removal systems of metal-based xenobiotics.

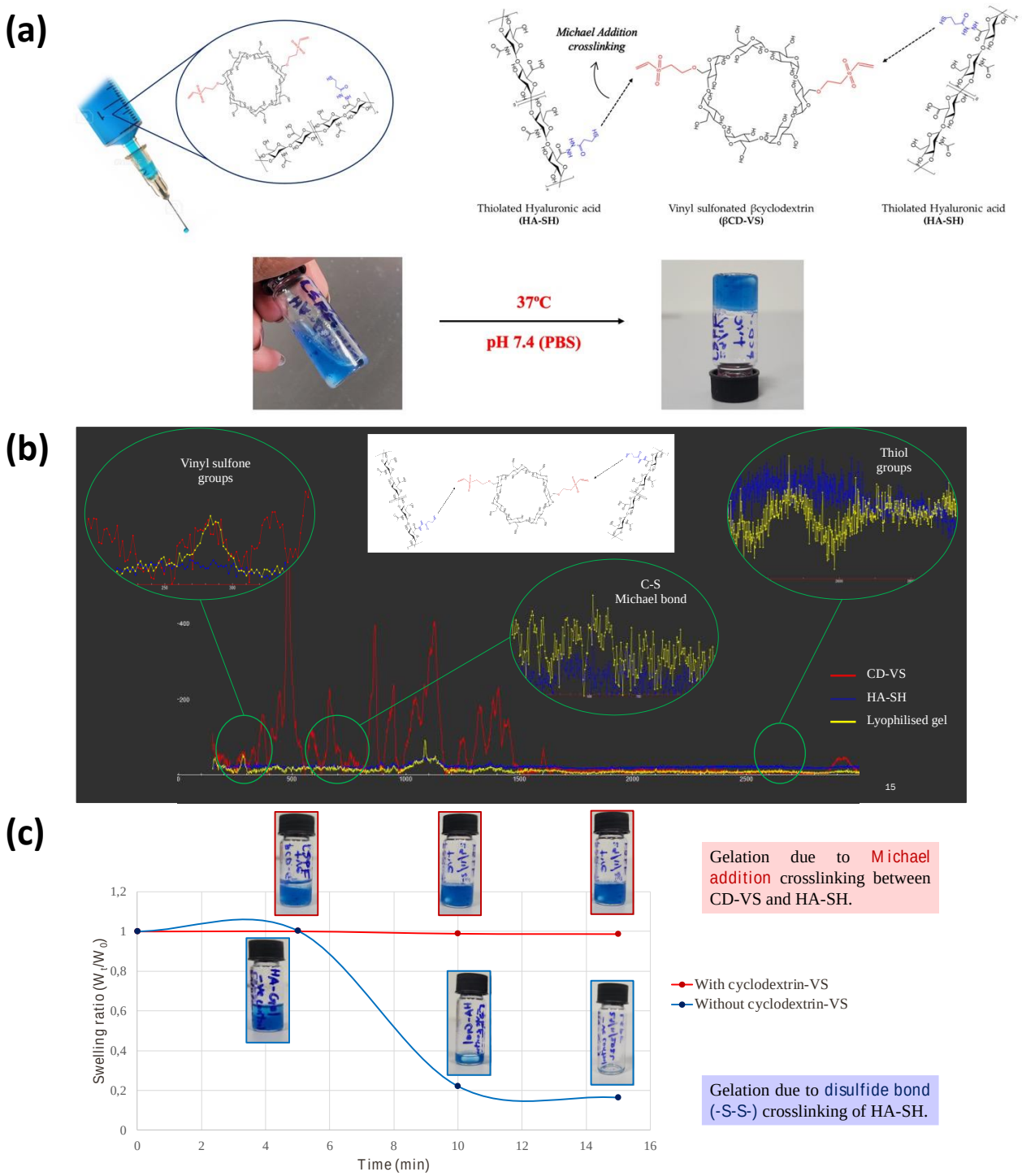

Figure 1. (a) Schematic gel formation via Michael addition crosslinking. (b) Combined RAMAN spectra of the system. (c) Stability demonstration of Michael addition crosslink under reductive conditions.

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Design and *in vitro* behaviour of niosome-loaded natural antioxidants for cosmetic use

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ABSTRACT

Among the natural antioxidant molecules, gallic and ferulic acids (GA, FA, Figure 1) are phenolic compounds with interesting properties, such as anti-inflammatory and antioxidant, for the treatment dermatological disorders[1,2]. However, their scarce water solubility and stability could be overcome by their encapsulation within delivery nanosystems obtaining suitable pharmaceutical or cosmetic formulations[3]. Specifically, this study was conducted to evaluate how the vesicular systems affect the release of the active ingredient and which formulation is most suitable for cutaneous application[4]. Niosomes, composed of Span 20 or Tween 20, were produced through the thin-layer hydration method using borate buffer or a poloxamer 188 micellar solution as the aqueous phase. Therefore they were characterized in terms of morphology (Figure 1), dimensional and encapsulation stability.

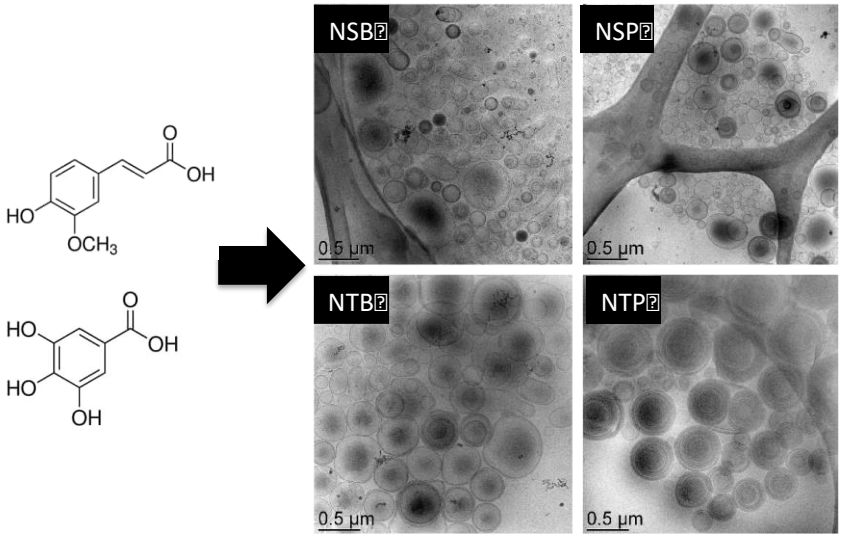

Figure 1. Chemical structure of GA and FA and cryo-TEM images of the produced niosomes

Xanthan gum and poloxamer 407 employed as thickening agents to obtain niogels with a certain grade of viscosity, spreadability and adhesiveness properties. The in vitro diffusion of drugs, studied by mean of Franz cells associated with membranes of mixed cellulose esters underlined the main role of poloxamer micellar hydration phase in governing the drug release from the niogels in ensuring the

controlled diffusion of polyphenols[5]. Lastly, the in vivo irritation test confirmed the safeness of niosomal gels after cutaneous applications.

Finally, gels based on poloxamer 407 or xanthan gum, were compared in terms of spreadability and adhesiveness indicating that gels based on poloxamer 407 have greater spreadability while those based on xanthan gum are characterized by higher adhesiveness.

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Inulin-g-branched polyethyleneimine and its amphiphilic analogue Inulin-g-branched polyethyleneimine-g-poly-D,L-lactide as siRNA delivery platforms

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ABSTRACT

siRNAs are double-stranded RNAs that act by targeting mRNA degradation within the cytoplasm. Despite their tremendous therapeutic potential, siRNAs face challenges such as poor in vivo stability and limited cellular uptake due to their high molecular weight, hydrophilicity, and negative charge [1]. To address these issues, the National Center for Gene Therapy and Drugs based on RNA Technology, a research network funded by the PNRR (Piano Nazionale di Ripresa e Resilienza) program, is dedicated to developing innovative siRNA delivery systems. Colloidal systems formed by the electrostatic interaction between negatively charged nucleic acids and positively charged polymers have emerged as a versatile solution.

In this study, we propose a novel library of graft semisynthetic polycations with diverse architectures and analogue compositions, designed specifically for targeted colonic delivery (Figure 1).

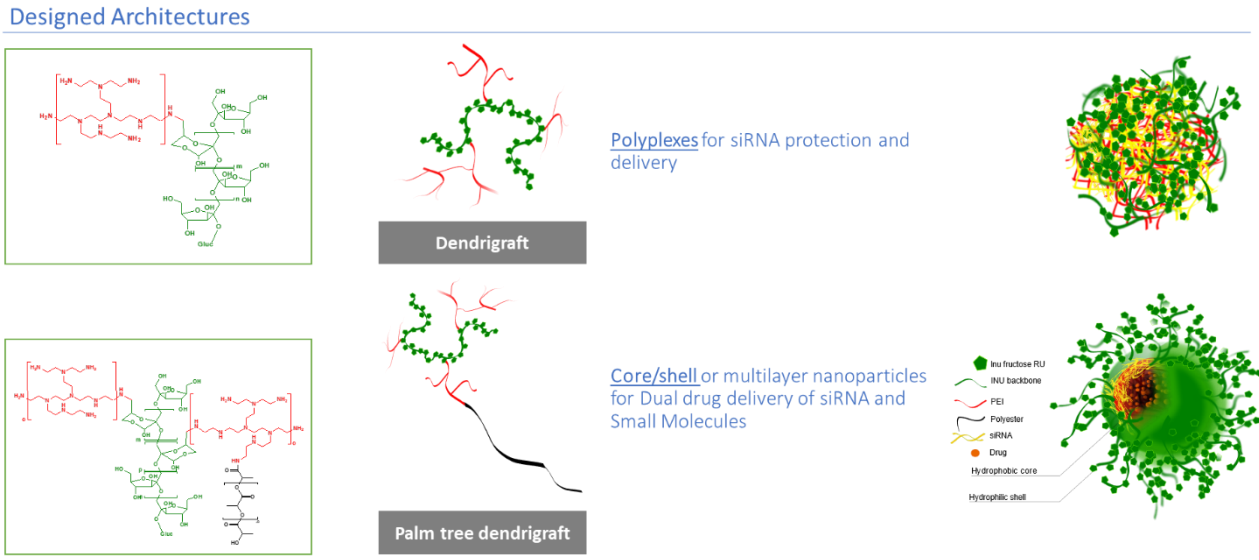

Figure 1. Designed inulin derivatives as a suitable platform for the development of a targeted siRNA delivery system

We selected inulin (INU), a natural polysaccharide, as the initial material based on previous investigations that demonstrated its suitability as a framework for siRNA vectors [2] and as a polyethylene glycol (PEG) alternative in amphiphilic derivatives for nanocostruction [3]. Moreover, INU has been used to develop dosage forms for colon targeting as it specifically degrades in the colon [4]. Here, we introduce the first derivatives of this series: Inulin-g-branched polyethyleneimine (INU-

bPEI) and its amphiphilic analogue, INU-bPEI-PLA. We synthesized derivatives with varying degrees of PEI derivatization and tested them to form copolymer/siRNA complexes through charge interactions. The successful formation of complexes was confirmed, and the nanosystems obtained were thoroughly characterized. Furthermore, we conducted preliminary biological studies that yielded promising results. Collectively, our findings provide encouraging insights into the development of efficient siRNA delivery systems, which can pave the way for improved therapeutic strategies.

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Formulation of α-tomatine-loaded lipid nanoparticles and evaluation of potential anticancer activity in an in vitro model of human neuroblastoma

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ABSTRACT

Tomatine (α-TM) is a steroidal glycoalkaloid extracted from stems, leaves and roots of tomato species which possesses numerous beneficial properties such as antioxidant, antiviral, anti-inflammatory, and anticancer [1]. Unfortunately, its potential therapeutic application is limited due to stability and bioavailability issues; thus, reducing the therapeutic effect of α-TM [2]. A widely used strategy for the delivery of natural compounds is their encapsulation into Solid Lipid Nanoparticles (SLN). 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 [3]. Therefore, the aim of this project was to develop an innovative nanoformulation containing α-TM (α-TM-SLN) for the potential treatment of cancer. Firstly, α-TM-SLN were prepared by *solvent-diffusion* technique and characterized in terms of mean diameter, polydispersity index (PDI), zeta potential (ZP) [4], differential scanning calorimetry (DSC) and long-term stability. Subsequently, the toxicological profile of α-TM was evaluated by the MTT assay on SH-SY5Y (human neuroblastoma cells) and OECs (olfactory ensheathing cells). The obtained results showed that α-TM encapsulated into SLN was able to reduce the viability of cancer cells more than free α-TM highlighting that the nanotechnological strategy has enhanced the therapeutic activity of the drug without interfering with cell vitality of healthy tissues. Therefore, the use of nanotechnology could be regarded as a promising strategy for delivering α-TM and exploiting its potential anticancer properties.

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Hydrogels from glycol chitosan as promising biocompatible scaffolds for wound repair

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ABSTRACT

A wound can be defined as a damage to anatomical structure of an organ that undermines its function. Treatment of acute or chronic wounds including pressure sores, surgical or traumatic wounds, where the risk of infections and complications are high, requires novel healing strategies [1].

Traditional dressings (e.g. gauze and cotton wool) do not provide appropriate antimicrobial activity for some types of wounds and have no active part in the wound healing process. Conversely, advanced dressings are designed to provide enhanced biological and antimicrobial activity based on peculiar properties of the same material or as a result of drug release.

In this context, the specific properties of hydrogels such as their biocompatibility, biodegradability, flexibility and hydrophilicity, make them good candidates to be used as wound dressing materials. To date, despite the great progress made, a lot of effort is still necessary to find an ideal system that combines high biocompatibility and ability to promote the wound healing process.

In a previous work, we prepared and characterized chitosan based scaffolds, starting from a chitosan derivative as the glycol chitosan (GCS), chemically crosslinked with diepoxy PEG (PEGDE) to give the GCS-PEG hydrogels. Preliminary results showed promising features for regenerative medicine application, as developed hydrogels displayed a good water affinity, a pronounced antibacterial activity, as well as pro-angiogenic activity [2].

This study aims at investigating the GCS-PEG hydrogels biocompatibility and their ability to promote the wound healing process. Indeed, a fibroblast cell line was used to test: 1) the biocompatibility of GCS-PEG hydrogels by evaluating cell viability 2) their effect on cell proliferation, and 3) cell attachment by optical microscope evaluation. Moreover, from an initial evaluation of the regenerative properties by means of a wound healing assay, the tested compounds appear to be promising medical device. The ability of the scaffolds to load and release a hydrophilic model drug gentamicin (GEN) was evaluated.

In order to optimize the formulation, rheological behaviour during and after the gelation phase, mechanical characterization in dry and swollen state as well as fracture mechanics testing methods, are being developed. Finally, the possibility of obtaining the GCS-PEG hydrogels with different shapes and

sizes (e.g., disks or sheets), also by varying their thickness, was evaluated to establish their ability to adapt to different potential medical applications for a future scaling up of these materials.

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PLGA-microplates by top-down low-pressure method: role of polymer on particles properties for drug delivery

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ABSTRACT

In the last decays, polymeric microparticles have found application in various biopharmaceutical applications, including controlled drug delivery systems. Poly(D,L-lactide-co-glycolide) (PLGA)-microPlates (μPLs) are polymeric microparticles already developed to deliver a wide range of payloads, characterized by square base of 20 × 20 ×10 μm height and homogeneous size, shape and surface area [2-3].

Employing a top-down fabrication methodology implemented under lower pressure protocols, novel μPL formulations were designed and characterized in terms of physio-chemical and biopharmaceutical properties. In particular, the role of PLGA on drug release mechanism was investigated varying the employed amount and using as model drug Curcumin (CURC), an anti-inflammatory and anti-cancer molecule [4].

Six different approaches were explored by valuing four PLGA masses, and three different purifications methods. The morphology of the μPL was defined by the Scanning Electron Microscopy (SEM), Fluorescence Microscopy, Confocal Microscopy, while Multisizer system was used to obtain size distribution and particle concentration. Drug encapsulation and release were determined by HPLC analyses (Fig.1).

Well-defined square-shaped particles were formed in all examined settings, while formulations with reduced PLGA mass had a noticeable concavity. The homogeneous fluorescent CURC emission indicated the uniform distribution of the drug in the particles. The achieved yields of the procedures were between 10-70%, while the CURC EE ranged between 2-13%, with the LE ranging between 0.1-5%. The CURC release profiles from each configuration were best matched with the Weibull empirical and the Korsmeyer-Peppas semi-empirical models, indicating that drug release was driven by Fickian diffusion or Anomalous Transport depending on the PLGA quantity. The best yielding and drug encapsulation values, as well as the slowest release rate, were obtained with the least quantity of PLGA, suggesting that polymer amount influences particle properties.

Finally, a unique and improved approach of fabricating PLGA-based microparticle drug delivery devices has been devised. This improved approach may enable the development of tailorable and adaptable systems appropriate for a variety of therapeutic applications.

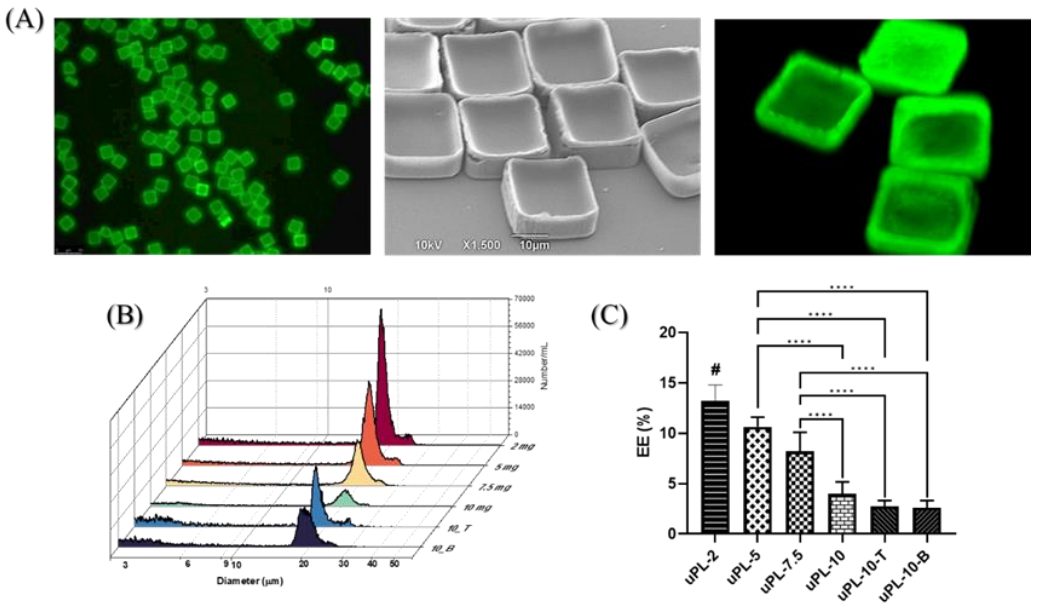

Figure 3: Geometrical characterization of µPLs with the higher PLGA mass: A) Fluorescence microscopy image (20X), SEM image, confocal microscopy z-stack (40X); B) Multisizer Coulter counter data; C) Encapsulation efficacy, with two-way ANOVA ($p<0.0001$; # µPL-2 significant with all groups)

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Effect of the pretreatment with microneedles on the skin penetration of cationic liposomes

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ABSTRACT

Skin delivery of drugs has been investigated for decades to achieve local or systemic effects. However, the strong barrier given by the tightly packed skin corneocytes prevents the passive diffusion of most compounds into the deepest strata. Among the approaches studied to breach the skin barrier, using microneedles (MNs) or very fluid and deformable liposomes seems to be the most effective; therefore, several studies recently proposed a combination of the two methods[1]. However, most of the research tracked the skin permeation of the drugs delivered with this approach, whereas the effect of MNs on the skin penetration of liposomes *per se* has been scantly investigated. In this work we produced and optimized microneedle arrays (MNA) with different geometrical properties to verify how the MNA pretreatment on the skin could affect the diffusion of cationic liposomes, either conventional rigid liposomes (RL) or fluid liposomes (FL).

Squared arrays of 6.4 mm^2 composed of 7x7 or 17x17 pyramidal MNs were produced with a 3D printer at different base diameters (150 or 200 μm), needle heights (600 or 1000 μm), and needles density (distance of 400 or 1000 μm). After UV curing (60°C, λ=405 nm), MNA were characterized for their morphology, mechanical strength (tensile testing machine) and skin depth (Parafilm model[2]). RL and FL liposomes were prepared by thin film hydration method. RL composition was DPPC/DOTAP/Cholesterol/Rho-PE 45/45/9/1 mol/mol/mol/mol whereas FL bilayers were composed of DOPE/DOTAP/Rho-PE 24/75/1 mol/mol/mol and Tween 80 was added as edge activator in 85:15 w/w ratio with respect to the lipid component. The particle size distribution and ζ potential were measured by dynamic light scattering (DLS). Low density MNA of 200x600 μm and 150x1000 μm were selected to evaluate the diffusion of RL and FL formulations into full-thickness porcine ear skin. Skin samples were poked with the MNA (30 N, 60 s), then placed onto Franz diffusion cells and treated with 50 μL liposomes dispersion under non-occlusive conditions for 15 min or 6 h before tape-stripping, heat separation[3], and observation under a fluorescence microscope.

MNs with distance of 1.0 mm reached at least 2 Parafilm layers; MNA 200 μm base and 600 μm height reached 4 parafilm layers (approx. 500 μm depth). A reduction in base diameter increased the fragility of 1.0 mm height MN, thus reducing the reached skin depth. Liposomes showed homogeneous nanometric size (121 ± 3 nm RL; 112 ± 2 nm FL) and ζ potential of 49 ± 10 mV and 28 ± 4 mV for RL and FL, respectively. Fluorescence imaging indicated that after 15 min FL could efficiently reach the epidermis/dermis interface (EDI) either with or without MN skin pretreatment, while the 200x600 MN improved the diffusion of RL to the EDI (ImageJ elaboration, $p<0.05$, Two-way ANOVA). Thus, MNs treatment may be required to enhance RL diffusion into the skin, albeit less impacting on FL diffusion.

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Inhalable Nano into Micro (NiM) particles as carriers for Rapamycin for the treatment of lung inflammation

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ABSTRACT

Based on the well-documented advantages of using inhalable nanomedicine for the local treatment of lung pathologies, in this work we describe the production of inhalable particles for the administration of Rapamycin for the treatment of inflammation-related lung diseases.[1] Rapamycin is a mTOR inhibitor agent, able to reduce pathogenetic processes such as accelerated inflammation and cell senescence.[2] In detail, lipid/polymer (hybrid) nanoparticles (LPHNPs) loaded with Rapamycin were produced starting from a fluorescent α,β-poly(N-2-hydroxyethyl)-DL-aspartamide (PHEA)/poly(lactic-co-glycolic) acid (PLGA) graft copolymer, and a lipid mixture between the 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and the 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-(polyethylene glycol)2000-Mannose (DSPE-PEG_{2000}-Mannose) to achieve the active targeting to alveolar macrophages.[3] Man-LPHNPs characterization demonstrated their nanometer size, a core/shell structure, the increased drug stability as well as a controlled release of the entrapped drug. By in vitro studies, the higher capability of the mannosylated LPHNPs to be internalised by macrophages respect to untargeted ones was also demonstrated. Then, the Man-LPHNPs were incorporated by spray drying (SD) into microparticles (Nano into Micro – NiM strategy) made by a mixture of polyvinyl alcohol (PVA) and leucin (LEU), optimising the aerosolization properties using ammonium bicarbonate as porogen. By starting from a liquid feed containing 5% wt/vol of PVA:Leu, 10 wt% of AB respect to PVA:Leu, and 1% wt/vol of Rapa@Man-LPHNPs, homogeneous microparticles were obtained with suitable properties to be inhaled as dry powder, such as high value of fine particle fraction (FPF%), and mass median aerodynamic diameter (MMAD) below 5 μm, capable to dissolve into the pulmonary fluid releasing the Rapa-loaded Man-LPHNPs, potentially up-taken by alveolar macrophages thank to active targeting (Figure 1).

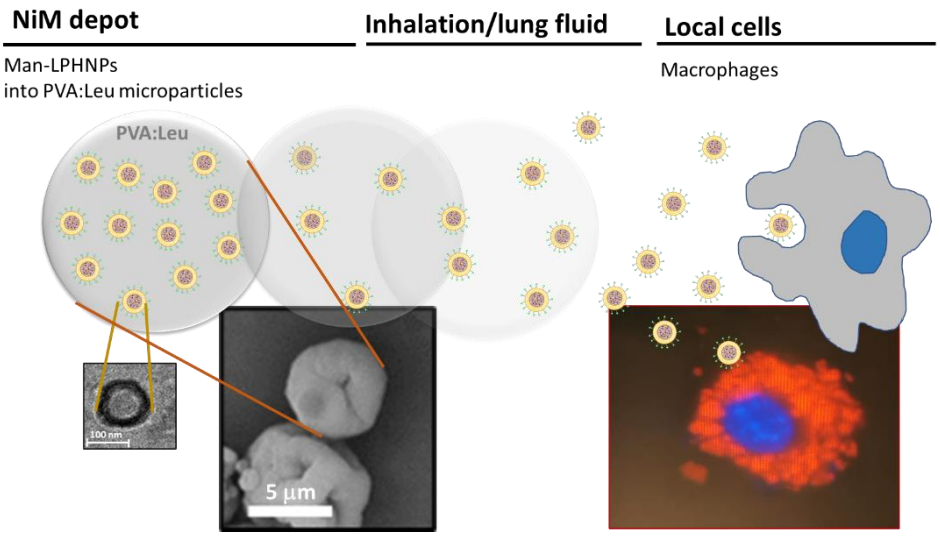

Figure 1. Inhalable NiM particles and the fate of the released Rapa-loaded Man LPHNPs.

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Polymer-coating of extracellular vesicles for a preferential delivery to hepatic stellate cells

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ABSTRACT

Extracellular vesicles of human mesenchymal stromal cells (MSC-EVs) are next generation therapeutics and were recently classified as biological drugs. Being cell-free agents, MSC-EVs recapitulate the pro-regenerative (anti-inflammatory, anti-oxidative and anti-fibrotic) properties of the parental cells, without the issues associated with conventional cell transplantation. In spite of the natural tropism of MSC-EVs for liver [1], challenges hampering the development of EV therapeutics do exist, and include the rapid clearance from the target site of systemically administered MSC-EVs.

We are developing polymer-based delivery strategies to improve the efficacy of EV-based therapeutics for non-alcoholic fatty liver syndromes, which are strongly associated with the onset of liver fibrosis. These strategies will ensure: 1) preferential delivery to hepatic stellate cells (HSCs); 2) protection from clearance; 3) sustained release. We first produced polymer-coated MSC-EVs with the aim to improve the targeting of HSCs. HSCs are liver cell types identified as a prominent source of collagen [2]. Once activated by chronic insults, HSCs switch their phenotype to collagen-producing myofibroblasts. Therefore, preventing/inhibiting HSC activation is one therapeutic strategy to alleviate/inhibit fibrosis progression.

The polymer of choice was hyaluronic acid (HA), a natural polysaccharide widely used in regenerative medicine and tissue engineering [3]. HA was selected due to consideration as a natural ligand of CD44 receptor, which is upregulated in activated HSCs [4]. We first synthesized a phospholipid HA derivative and verified its binding to HA by nuclear magnetic resonance (1H-NMR). Then, the obtained polymer was labeled with a fluorescent probe and used to coat MSC-EVs.

Preliminary characterization of polymer-coated MSC-EVs by flow cytometry, nanoparticle tracking analysis (NTA) and confocal microscopy revealed a stable fluorescent product with no modification of EV size (approx. 150 nm diameter). Interestingly, the fluorescent signal detected in the cytosol of target cells 6-hour after adding the MSC-EVs suggested their successful cellular uptake. Studies toverify biological activity (cell-based assays) and stability (Z potential) of polymer-coated MSC EVs were also performed. These newly synthesized fluorescent polymer-coated MSC-EVs could be also

exploited to launch future in vivo biodistribution studies. Design and synthesis of injectable hydrogel laden with MSC-EVs is ongoing. The hydrogel might sustain the release MSC-EVs to liver, also reducing the need for multiple administrations.

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A side-by-side comparison of lipid and polymeric nanoparticles for (DDC)2-Cu-based cancer therapy

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ABSTRACT

Disulfiram, an anti-alcoholism drug approved by FDA in 1951, has demonstrated a remarkable anticancer activity,[1] mainly due to its metabolite diethyldithiocarbamate-copper complex ($(DDC)_2$-Cu) .[2,3] However, $(DDC)_2$-Cu short half-life, lack of selectivity and low water solubility, restrict its efficacy. Thus, $(DDC)_2$-Cu encapsulation in drug delivery systems may represent an efficient strategy to overcome these limitations.3 Herein, PLGA-PEG2kDa polymeric nanoparticles (PNPs) and solid lipid nanoparticles (SLNs) loaded with $(DDC)_2$-Cu were formulated by nanoprecipitation and microfluidic device, respectively. PNPs and SLNs of 172.1 ± 8.1 nm and 106.9±5.79 nm, with a PDI of 0.035±0.02 and 0.103±0.03, respectively, were obtained, according to DLS measurements (Fig 1A, B). The zeta-potential was almost neutral for both formulations, while TEM analyses showed a spherical shape and a smaller size in comparison to DLS, with 92.7±1.72 nm for PNPs and 82.62±0.03 nm for SLNs. PNPs had a much higher encapsulation efficiency percentage (EE%) as compared to SLNs (60.9±16.5% and 7.22±1.22%, respectively) and a better stability when stored in PBS at 4°C. Furthermore, they did not interact with serum proteins for at least 48 h when 5% FBS was added. On the contrary, SLNs were unstable in PBS, yet serum proteins interaction with their surface prevented this phenomenon. Release studies performed at 37°C showed that 23% of $(DDC)_2$-Cu was freed from PLGA-PEG2kDa NPs within the first 8 h, and more than 50% of the encapsulated drug in the following 10 days of monitoring. When incubated at 37°C in aqueous environment for 48 h, SLNs did not release $(DDC)_2$-Cu, suggesting that the drug is strongly retained in the solid bulk of the SLNs. Finally, free $(DDC)_2$-Cu and $(DDC)_2$-Cu-loaded PNPs and SLNs were tested for their anticancer activity against MDA-MB-231 and MCF-7 breast cancer cell lines (Fig 1C, D). In all experiments, both free $(DDC)_2$-Cu and loaded nanocarriers showed a concentration-dependent cytotoxicity and low IC50 values. In MDA-MB-231 cell line, PNPs showed slightly higher activity than SLNs (IC50 values of 0.23 and 0.28 μM, respectively), and comparable to that of the free drug. Instead, in MCF-7 cell line PNPs and SLNs were more active than free $(DDC)_2$-Cu (IC50 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 efficacy.

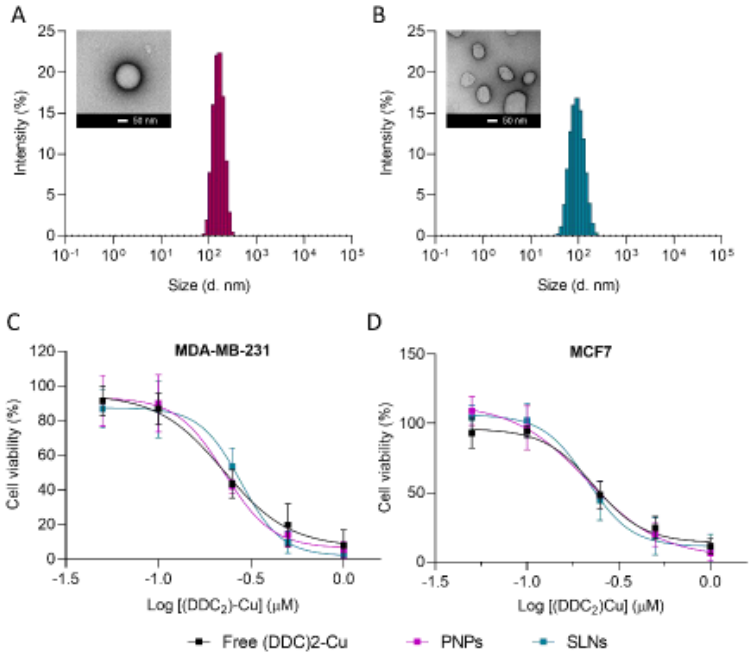

Figure 1. DLS and TEM analysis of $(DDC)_2$-Cu-loaded PNPs (A) and SLNs (B); cytotoxicity studies in MDA-MB-231 (C) and MCF7 (B) cells.

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Phycocyanin gastro-resistant microparticles produced by spray-drying for the treatment of Inflammatory Bowel Diseases

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ABSTRACT

Inflammatory bowel diseases (IBD) like Crohn's disease and ulcerative colitis involve inflammation in the digestive system due to an autoimmune response. Conventional treatments often have limited effectiveness and adverse effects, prompting the search for natural compounds with antioxidant and anti-inflammatory properties as alternative therapies [1]. Phycocyanin (PC), a water-soluble protein found in algae, has gained attention for its antioxidant and anti-inflammatory properties. PC contains a unique structure with phycocyanobilin (PCB) chromophores covalently linked to the protein via thioether bonds. However, PC stability is strongly affected by pH and temperature. Destabilization of PC leads to its degradation and changes in spectral characteristics and color, which are notably affected by the acidity of the environment [2].

In this work, a microencapsulation technique such as the spray-drying technique was used in order to produce gastro-resistant microparticles (MPs) as controlled release systems for PC. The study aimed to develop a novel pharmaceutical formulation using natural excipients such as soy proteins (SPs) that could provide technological advantages and ensure the stability of the active ingredient, PC, which is sensitive to acidic conditions. Scanning electron microscopy (SEM) images showed a quite homogeneous spherical shape of the MPs, with an average diameter between 5 and 15 μm. FTIR analysis was used to evaluate the effective incorporation of PC within the microparticles and the absence of any degradation to the components of the formulation. The differential scanning calorimetry (DSC) comparison graphs showed that there are physical interactions between SPs and PC in the solid produced by the spray drying process. To evaluate the antioxidant capacity of the MPs at different concentrations, DPPH assay was performed to study their free radical scavenging properties. Then, an ex-vivo permeation study through porcine colon mucosa showed a higher transmucosal permeation capacity for the MPs produced compared to the pure active ingredient with a clearly gastro-resistant release profile.

Moreover, in vitro studies using NHDF as model cells have demonstrated that the MPs are totally cytocompatible at different doses; moreover, they appear to have a cytotoxic effect on Caco-2 cells. In conclusion, in vivo studies on a rat model of IBD are currently underway to assess the actual ability of the PC and the new MPs produced to exhibit a protective and/or curative effect against IBD in an animal model through anti-inflammatory and antioxidant-like action.

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Synthesis and characterization of novel redox-sensitive waterborne polyurethanes

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ABSTRACT

Waterborne polyurethanes (WPUs) in recent years, attained a more increasing attention due to their environmentally friendly nature and water dispersibility. Unlike traditional organic solvent-based polyurethanes, WPUs are synthesized using water. The synthesis involves the reaction of diols, such as polyether or polyester diols, with diisocyanates and chain extenders in presence of a suitable emulsifier. The emulsifier enables the dispersion in water of the hydrophobic polyurethane segments, resulting in a stable colloidal system[1]. These versatile polymers offer a wide range of applications, including coatings, adhesives, sealants, and biomedical materials[2] even in the biomedical field, e.g., drug delivery systems (DDS), tissue engineering scaffolds, and biomedical coatings. The capability to incorporate bioactive molecules and to control drug release makes WPUs promising materials for these applications. In this work, novel redox-sensitive WPUs based on a PCL-PEG-PCL triblock copolymer[3], 2,2-bis(hydroxymethyl)propionic acid (DMPA) as emulsifier, isophorone diisocyanate and L-glutathione oxidized as a redox-responsive chain extender were synthesized (**Fig.1a**). Using different PCL-PEG-PCL/DMPA molar ratios (0.45, 0.25 and 0.15), three WPUs with increasing extent of hydrophilic character were prepared. The synthesized WPUs were characterized by FTIR, ^{1}H-NMR, SEC and DSC. The nanoparticle sizes of the aqueous WPUs dispersions, their dimensional distribution and Z potential were investigated. Considering to PCL-PEG-PCL/DMPA molar ratios, as DMPA content increases particle size decreases due to the higher amount of carboxylic groups. The properties and the behaviour of such WPUs, including their hydrolysis pattern and the hydrophilicity grade, were investigated. It was observed that the hydrolysis rate rises as the quantity of DMPA in WPUs increases. The presence of different PCL-PEG-PCL/DMPA molar ratios affects the degree of swelling, according to the increase of DMPA.

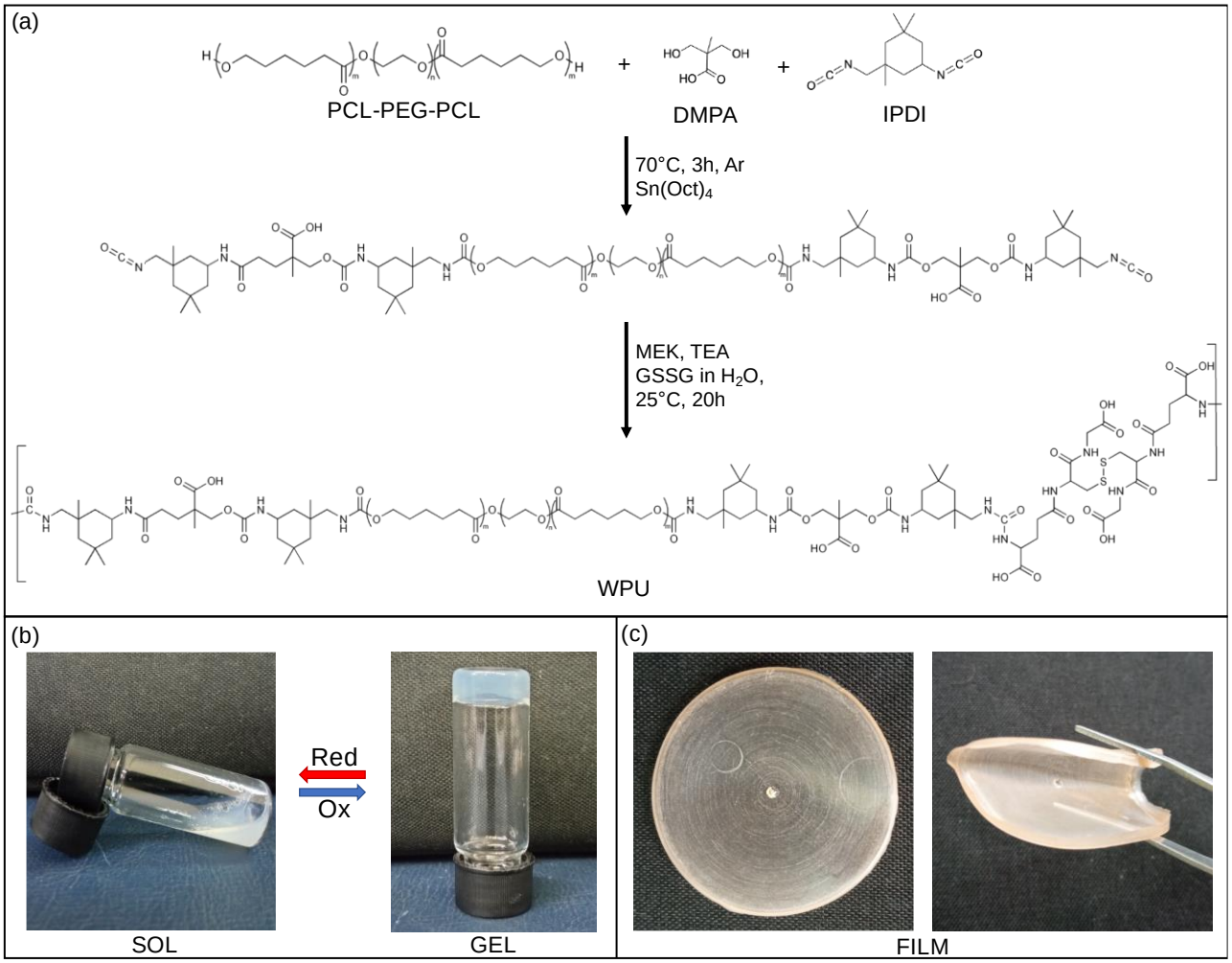

Figure 4: WPU: a) scheme of synthesis; b) dispersion before and after oxidation; c) film obtained by film casting.

WPUs were processed to obtain hydrogel and film (**Fig.1b-c**). Interestingly, starting from lyophilized samples, through autoclaving process, aqueous dispersions of WPUs were easily produced. These aqueous dispersions were able to undergo a reversible sol-gel transition in response to external oxidative/reductive stimuli. Furthermore, the capability of WPUs to release drug from hydrogel and film was investigated. This feature opens the way for the development of WPU-based redox responsive DDS with controlled release capability for the locoregional treatment of solid tumours.

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A NEW MUCOSAL PENETRATING PEPTIDE TO TARGET LIPOSOMES TO THE ESOPHAGOUS

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ABSTRACT

Esophageal local pathologies affect hundreds of millions of people a year worldwide. However, the delivery of drugs to the esophagus is very challenging because of the quick clearance from saliva, the low transit time (less than 15 minutes) and the barrier properties of esophagus itself that limit drug penetration in the mucosa. To overcome the issue of the short residence time, mucoadhesive dosage forms, drug-eluting stents and shape memory dosage forms have been designed, while little efforts have been made to increase drug penetration in the mucosa 1. In this work, surface-modified liposomes with novel mucosa penetrating peptides were designed to improve both the residence time and drug penetration in the esophagus. Mucosa penetrating peptides were screened by phage display using fresh porcine esophagus as a membrane. The selected peptides were synthesized by microwave assisted solid phase peptide synthesis using Fmoc-chemistry adding a cysteine residue at C-terminal for the further conjugation to liposomes. Liposomes compositions were the following: DPPC:DOPC.CHOL 60:30:10 mol%; DPPC:DOPC:CHOL:DSPE- PEG_{2000}NH2 60:30:9:1 mol%; DPPC:DOPC:CHOL:DSPE-PEG2000MALEIMIDE mol%; DPPC:DOPC:CHOL:DSPE-PEG2000MALEIMIDE 60:30:5:5 mol%. Liposomes were loaded with curcumin (CUR) that is indicated in the treatment of the esophagitis. Ex vivo permeation experiments were carried out using Franz diffusion cells and porcine esophagus. Three peptide sequences were found in more than 20% of clones, namely NPLLLRG (P1), QWQGSVW (P2), and SLENKGP (P3), whose N-Cys derivatives were conjugated on liposome surface via maleimido chemistry. Liposomes with a hydrodynamic diameter ranging between 80 and 100 nm were obtained in all cases and the conjugation efficiency of the peptides was always higher than 70%. As expected, pegylation slightly favoured the penetration of CUR in the esophagus compared to not pegylated control liposomes because of the intrinsic weak mucus penetrating properties. Nevertheless, only P3-decorated liposomes significantly enhanced CUR retention in the esophagus. Furthermore confocal microscopy studies revealed that P3 decorated liposomes can reach the deepest layers of the esophagus mucosa already after 5 minutes application whereas pegylated liposomes remained stuck to the mucosal layer (**Figure 1**). Finally, after oral administration in healthy mice (species with a very short esophageal transit), it was found interestingly that pegylated liposomes remained in the esophagus for at least 15

minutes, regardless of the presence of the peptide. However, P3 seemed to accelerate the interaction of liposomes with the tissue wall and further partitioning in the cells.

In conclusion, the study provides a preliminary indication for the use of mucosa-penetrating peptides as a promising strategy for drug targeting to the esophagus.

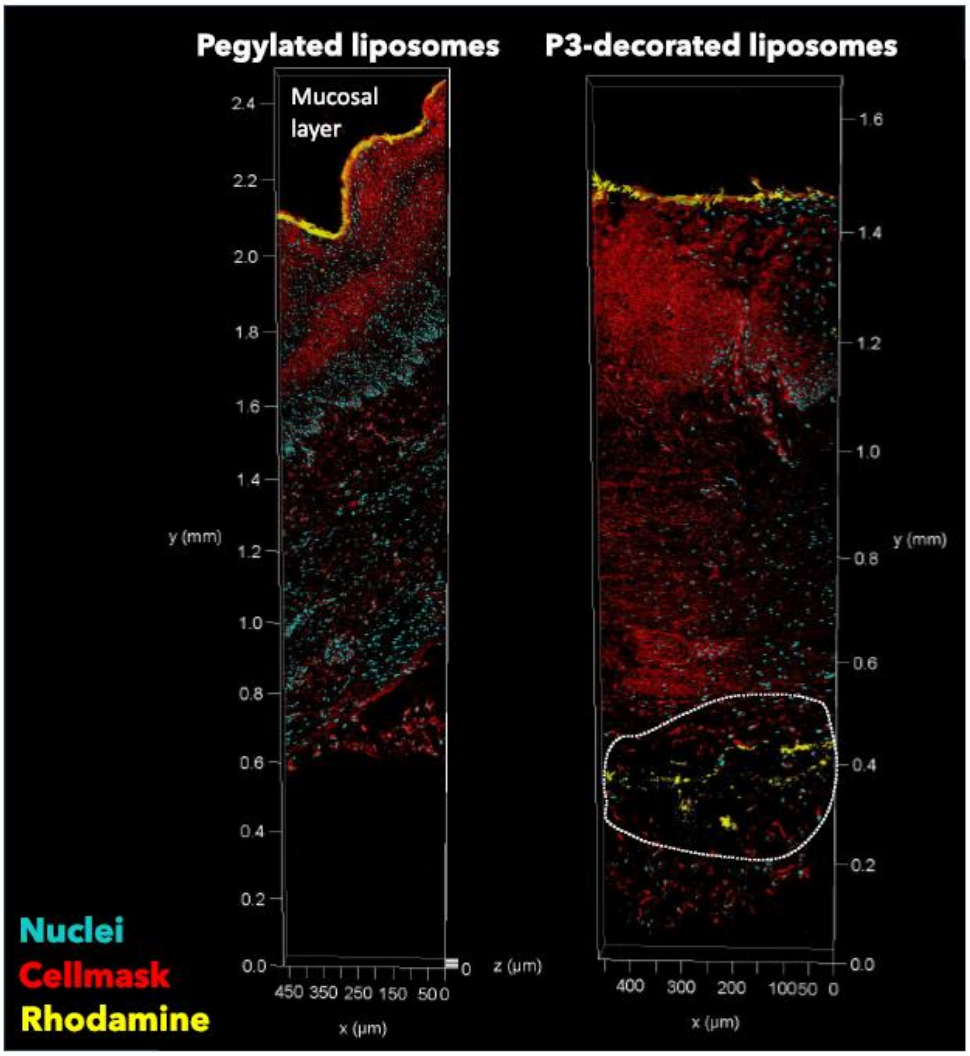

Figure 1- Confocal imaging of esophagus tissue treated with rhodamine (yellow)-labelled pegylated or P3-decorated liposomes

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Keratin nanofibers-based patches loaded with pomace extracts for wound healing applications manufactured via solution blow spinning technology

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ABSTRACT

Recently, patches constituted of interwoven keratin nanofibers have gained increasing interest in wound healing applications due to the mucoadhesive and bioresorbable properties of the protein. Although electrospinning (ES) technology is a well-known method for creating these nanostructured patches, the in-situ deposition of the keratin nanofibers is constrained due to the usage of high voltages. In comparison to ES, the Solution Blow Spinning (SBS) technology provides several advantages, such as cheaper spinning apparatus costs, easier industrial scale-up, and the potential for in-situ deposition of the patch (such as a laparoscopic deposition).

In this study, the feasibility of processing High Molecular Weight-Keratin (HMW-K) aqueous solutions into nanofibers by the SBS approach was investigated for the first time, with the final goal of manufacturing regenerative skin patches loaded with a pomace extract endowed with antimicrobial activity.

For this purpose, HMW-K/Polyethylene oxide (PEO) 900 KDa aqueous solutions were successfully spun into nanofibers-based patches using a self-assembled SBS apparatus. The effect of different ratios of HMW-K/PEO 900 and critical process conditions was studied on the deposited matrices' morphology using the Design of Experiment (DoE) approach. Scanning Electron Microscopy (SEM) images revealed that matrices produced with the selected optimal process parameters were made up of an interweaving of nanofilaments without defects (Fig. 1). Moreover, to reduce the water-solubility of the collected samples, the addition of citric acid (CA) as a cross-linker to the HMW-K/PEO aqueous solution was investigated through rheological measurements, water-stability studies, and Fourier-transform infrared spectroscopy (FT-IR) analyses. After cross-linking, the HMW-K/PEO/CA solutions attained suitable zero-shear viscosity and polymer chain relaxation time values. Finally, in vitro drug release studies of the resulting pomace extract-loaded patches were performed in a PBS medium, and the patches showed a sustained release of the drug for 48 hours.

Hence, the SBS methodology was effectively used for the first time to process keratin-based aqueous solutions, offering a potential method in the field of biomedicine for the in-situ production of patches for wound healing applications.

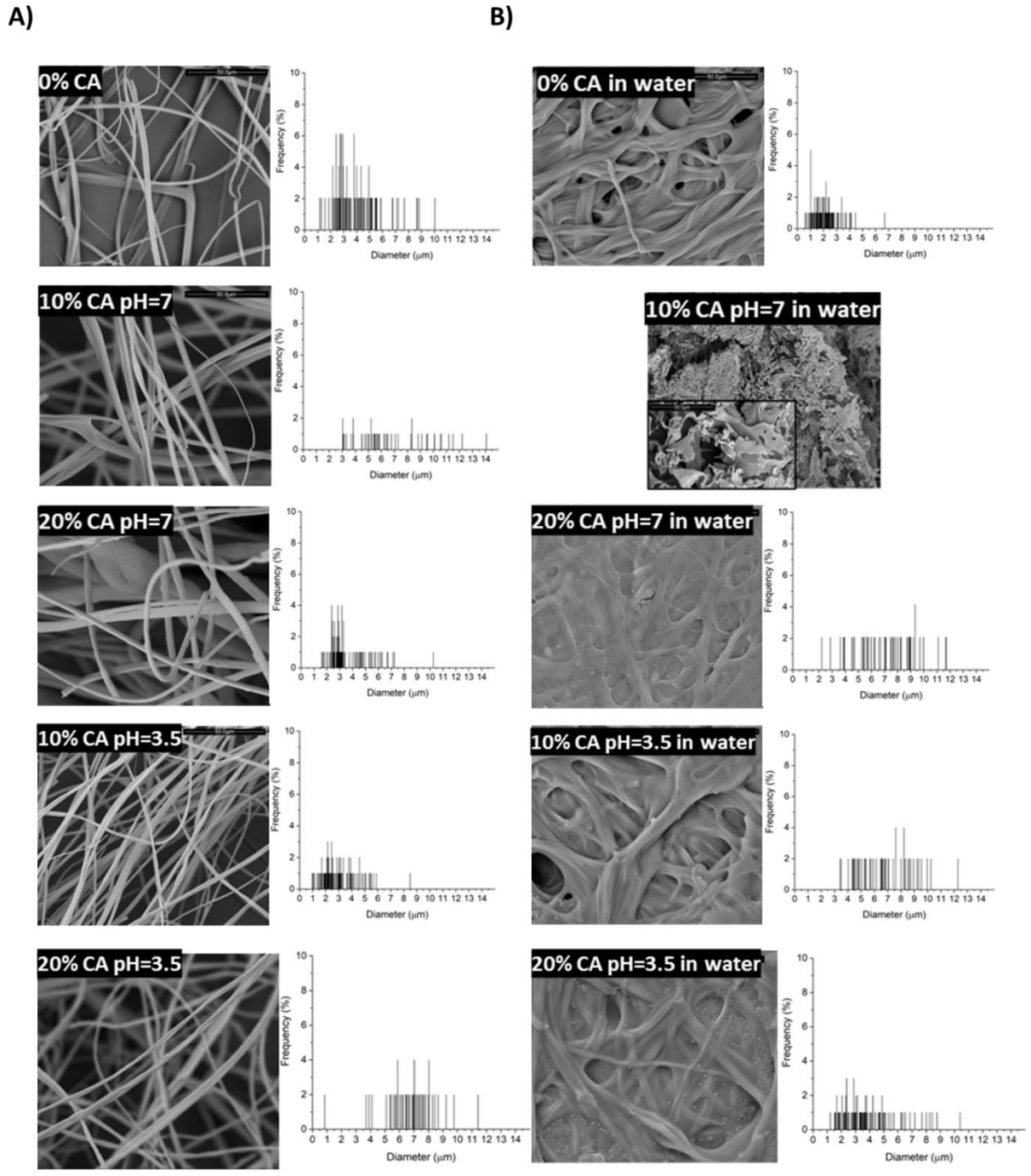

Figure 5. SEM images and diameters distribution of electrospun fibers pre- and post-crosslinking process A) untreated and B) annealed at 150° C for 5 minutes and immersed in water.

Rutin-loaded zein gel: A novel biocompatible green wound dressing

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ABSTRACT

Wound management is one of the most pressing clinical issues because millions of people experience various types of wounds and injuries. A suitable dressing should be applied to the wound to protect it from external mechanical and microbial stress [1]. In this scenario, zein protein has shown to be an appealing material for wound healing applications due to its unique set of biological properties, which include non-toxicity biocompatibility, biodegradability. Moreover, the protein has shown hemostatic and mucoadhesive properties which could be used to accelerate the wound healing [2,3]. Based on these findings, the goal of this study was to assess the effect of different amounts of rutin on the rheological properties of zein-based gels using passive and dynamic rheology in order to develop an innovative biocompatible dressing. The samples were characterized by a decrease in SLB and higher EI when the rutin concentration was increased, showing a viscoelastic behavior. The increase of rutin concentration showed frequency-dependent profiles up to 2.5% w/w of the active compound while the use of 5% w/v of drug showed that both moduli of the gels are completely frequency-independent, highlighting a shear-thinning behavior. The drug leakage from the zein gels in a PBS solution was inversely proportional to the concentration of the active compound; in particular, the formulations prepared with 5% of rutin showed a sustained release up to 160 h. It's interesting to observe that the empty gels promoted a faster migration and proliferation of keratinocytes after 24 h and 48 h when compared to the control as demonstrated by the high wound closure rates (~ 83% and 93%, respectively). The encapsulation of rutin within the gels improved the cell migration showing a significant reduction in the scratch area after 24 h incubation (~ 90%) and a complete closure after 48 h (Figure 1) . Furthermore, a full thickness excision was created on the backs of Wistar rats, and the various samples were applied daily on the wound sites to assess the *in vivo* wound healing. Among the different formulations, the zein-based gels containing rutin promoted the most rapid wound contraction; in particular, at day 10 a decrease of the wound area of about 90% was obtained with respect to the saline control and the other samples (Figure 1). Moreover, rutin-loaded zein gel showed a remarkable down-regulation of the levels of IL-1β, IL-6,

TNF-α and an increase of IL-10 concentration as compared to untreated groups (all $p<0.05$). These features open new perspectives concerning the conceivable application of rutin-loaded zein gels in the treatment of various types of wounds or lesions.

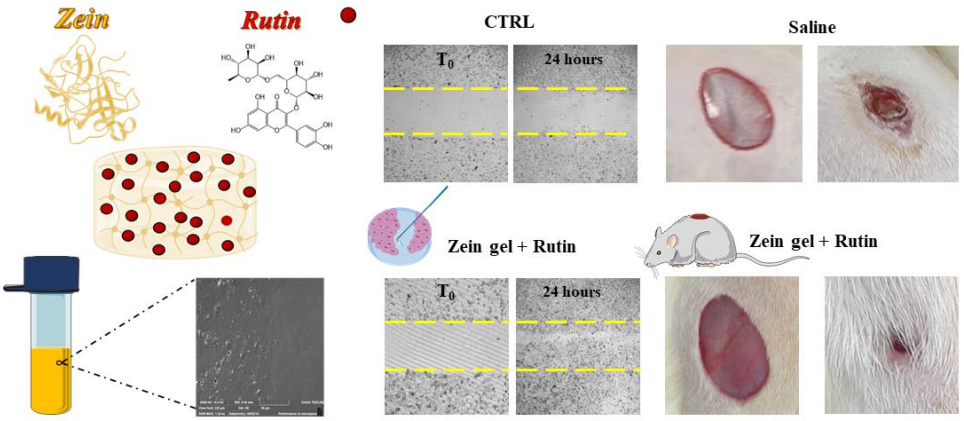

Figure 1 – *In vitro* and *in vivo* wound dressing activity of rutin-loaded zein gels.

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Enhancing PCL for 3D Printed Bone Scaffolds: Novel Strategies to Overcome Limitations

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ABSTRACT

Tissue-engineered scaffolds have revolutionized tissue repair, particularly in bone tissue regeneration[1]. This study explores the potential of 3D printing (3DP) techniques, specifically extrusion-based methods, to enhance the performance of bone scaffolds using Polycaprolactone (PCL). Fused Filament Fabrication (FFF) and Semi Solid Extrusion (SSE) techniques offer versatility, precision, and cost-effectiveness[2]. However, the limited selection of printable biomaterials remains a challenge.

PCL, a biodegradable synthetic polyester, has gained attention as a bone scaffold material due to its biocompatibility, low cost, FDA approval, and ease of processing[3]. To improve PCL properties, we incorporated both organic and inorganic components, optimizing its hydrophilicity and bioactivity. Dexamethasone, an osteoconductive agent, was added to promote bone healing[4].

We propose two strategies for producing advanced 3D printed bone scaffolds: (1) developing PCL-based hybrid materials by blending with organic (e.g., alginate, microcrystalline cellulose, or inulin-grafted-poly(D,L)lactic acid grafted copolymer[5]), and inorganic-components (e.g., nanohydroxyapatite), and (2) anchoring bioactive substances on the PCL surface. We functionalized PCL with pendent ammino groups for bioconjugation with Arg–Gly–Asp (RGD), enhancing cell-material interaction.

Using FFF and SSE techniques, we 3D printed macroporous scaffolds with various hierarchies and nanotopographies. Physicochemical and technological properties, including size, morphology, mechanical strength, and degradation, were evaluated. Preliminary biological studies confirmed scaffold biocompatibility and mechanical integrity, while improved properties such as wettability, surface roughness, swelling ability, and biodegradation were achieved through blending and surface modifications.

This study highlights the relationships between biomaterial arrangement and fabrication strategy, leading to the successful development of bone scaffolds. The ability to modulate hierarchy, nanotopography, and drug release mechanisms within the scaffold structure holds significant implications for personalized bone fracture regeneration.

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Influence of capsule size and DPIs resistance on inhalable dry powder aerodynamic performance

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ABSTRACT

Dry powder inhalation therapy is recognized to be the most widely used strategies for targeting pulmonary diseases. To ensure high bronchial deposition of the drug, powder physio-chemical characteristics, namely particle size distribution, particle density and shape or morphology must be analyzed. However, the inhalation device design play a crucial role in lung deposition. Inhalation therapy, in fact, is known as a combination product as it consists of a formulations of API and excipients combined with the device for delivery, such as dry powder inhaler (DPI)[1].

The pre-metered single-unit dose DPIs, working with HPMC capsules as powder reservoir can offer an easy design controllable characteristics such the size of capsules, and consequently, the filling volume. Additionally, the inspiratory flow rate generated by the patient's inspiratory effort creates a negative pressure depending on the devices resistance[2].

The aim of this project was to study the influence of different HPMC capsule sizes, device resistance and mouthpiece shape on the aerodynamic performance, of a nano in microparticle spray dried powder. Emitted Fraction (EF%) and Fine Particle Fraction (FPF%) of the inhalation micropowder have been assessed and compared among differently designed devices.

The DPIs was RS01 (Plastiape®), in which the dose emission mechanism activated by the patient inhalation act consists in capsule lifting and rotating inside the device body and centrifugal emission of the powder. RS01 with capsules size 3 (0.30 mL) or 2 (0.37 mL) and flow rates of 65 L/min or 80 L/min or different size of mouthopiece with capsule size 3 (0.30 mL) and flow rate 65 L/min were selected. The aerodynamic tests were performed by Fast Screening Impactor (FSI) with gravimetric quantification method.

By comparing the aerodynamic performance between the devices mentioned, the FPF % resulted significantly increased using RS01 size 2 for both flow rate 65 L/min and 80 L/min compared to the results obtained with capsule size 3 at 65 L/min. This is probably do to the larger fill volume of capsule size 2 compared to size 3 which allow a better aerosolization of fine particle powder. In addition, the device having a large cylindrical mouthpiece shows an important increased FPF% compared to RS01, size 3, 65 L/min. Finally, the different flow rate in the RS01 device, size 2, 65 L/min didn’t show a significant difference in terms of FPF% in comparison with the RS01 same capsule size and higher resistance.

In conclusion, these results demonstrated that the inhalation device design plays a crucial role in the lung deposition of a dried powder, especially in terms of capsule size.

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Development of flurbiprofen-loaded PLGA microspheres using the membrane emulsification approach

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ABSTRACT

Poly(lactide-co-glycolide) (PLGA)-based microspheres (MS) represent the majority of the biodegradable long-acting injectables (LAIs) administered by subcutaneous route. During formulation optimization and development of copies, several variables (*i.e.,* type and origin of PLGA and the manufacturing process) can affect the size distribution and the microstructure of MS, thus impacting the biopharmaceutical performances.

Drug release studies are often inadequate for screening the effect of both formulation and process parameters since conventional buffers do not take into consideration the quali-quantitative composition of interstitial fluids (IF) in subcutis in terms of macromolecular components, osmolarity, and pH which can influence the MS surface and drug diffusion. Therefore, this study aims to provide preliminary data on the development of a drug release test using biorelevant media. To this end, MS prepared using two types of PLGA were loaded with flurbiprofen, selected as a model lipophilic drug. MS were produced through the membrane emulsification/solvent evaporation method (Micropore Technologies Ltd, UK) and formulation parameters were optimized to achieve a target size of 50 μm with a narrow size distribution. Drug loading and encapsulation efficiency (EE%) were assessed by HPLC. The release tests were carried out in four biorelevant media prepared based on literature data on the composition of the SC interstitial fluid and differing in terms of osmotic/oncotic pressure, viscosity, and the presence of albumin and hyaluronic acid, either individually or in combination. The release assay was conducted statically at 37.0 ±0.5 °C for four weeks.

MS with D50 = 30-70 μm and the highest yield were achieved by setting the injection speed at 0.5 ml/min, the stirring rate at 4V, using 12% (w/v) PLGA and 2% (w/v) HPMC as a stabilizer. The drug loading was approximately 12% and EE was 60%, regardless of the type of PLGA used, indicating that membrane emulsification/solvent evaporation is a suitable technique for achieving a narrow size distribution and a good EE%.

As expected, the release of flurbiprofen from the MS made of capped PLGA was slower compared to uncapped PLGA. The presence of macromolecules significantly influenced the drug release profile (*Figure 1*). Specifically, albumin increased the rate of drug release, while hyaluronic acid slowed it down compared to the saline solution alone. These differences were particularly evident for MS made of capped PLGA.

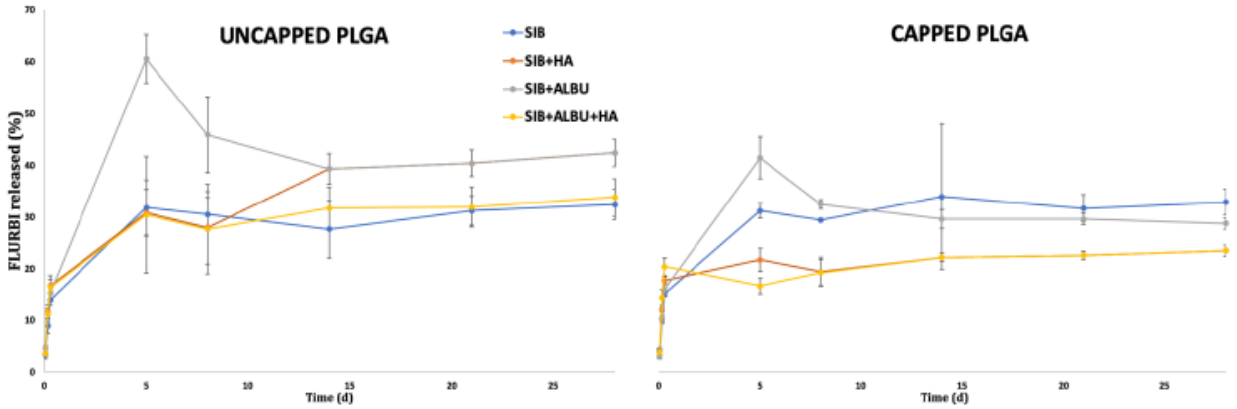

Figure 1- flurbiprofen released from PLGA microspheres in simple buffer alone (SIB) or in buffer containing hyaluronic acid (SIB+HA), albumin (SIB+ALBU), or both macromolecules (SIB+ALBU+HA).

In conclusion, the composition of the dissolution medium, especially the presence of proteins, plays a crucial role in influencing the release profile of flurbiprofen from PLGA microspheres. These findings underline the relevance of using release media that closely mimic the SC tissue environment.

Electrosprayed Poly-butyl-succinate microparticles for sustained release of Ciprofloxacin

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ABSTRACT

In recent decades, the field of local therapy for the treatment of infections has made significant advancements through the use of controlled drug delivery systems. The treatment of chronic infections, particularly those caused by antibiotic-resistant bacteria, poses significant challenges. Polymeric microparticles can offer a valuable solution to improve the efficacy of antibiotics in the treatment of antibiotic-resistant infections, as they are able to load drugs while modifying their release profile, improving efficacy of the treatment.[1]

Here we present a study on the characterization and evaluation of antibacterial properties of electrosprayed microparticles of polybutylene succinate (PBS), a biodegradable and biocompatible polyester, which were loaded with ciprofloxacin (CPX), a fluoroquinolone antibiotic, inhibiting bacterial replication and effectively treating various infections (Fig.1). PBS is a well-known water-insoluble polymer with tuneable chemical-physical properties, therefore is well suited to tissue regeneration or wound healing applications.[2]

The versatile and user-friendly electrospraying technique enables successful incorporation of the poorly water-soluble broad-spectrum antibiotic CPX into a polymeric matrix. This drug delivery system allows sustained release of CPX for up to 6 days. An ex vivo permeation study on porcine skin, serving as a model for human skin, evaluated the drug permeation to assess potential enhancement in drug permeation. The microparticles were characterized using SEM-EDX, ATR-FTIR, DSC, and TGA, and their degradation rate was tested in DPBS and human plasma.

Then to evaluate the antimicrobial properties of the drug delivery system, CPX-loaded microparticles were evaluated against common pathogens such as *S. aureus* and *P. aeruginosa*, including skin bacteria that hinder wound healing and promote chronic wound development. MIC and MBC assays were conducted using different culture media. Effective antibacterial activity was observed, along with inhibition of P. aeruginosa biofilm formation at sub-MIC concentrations.

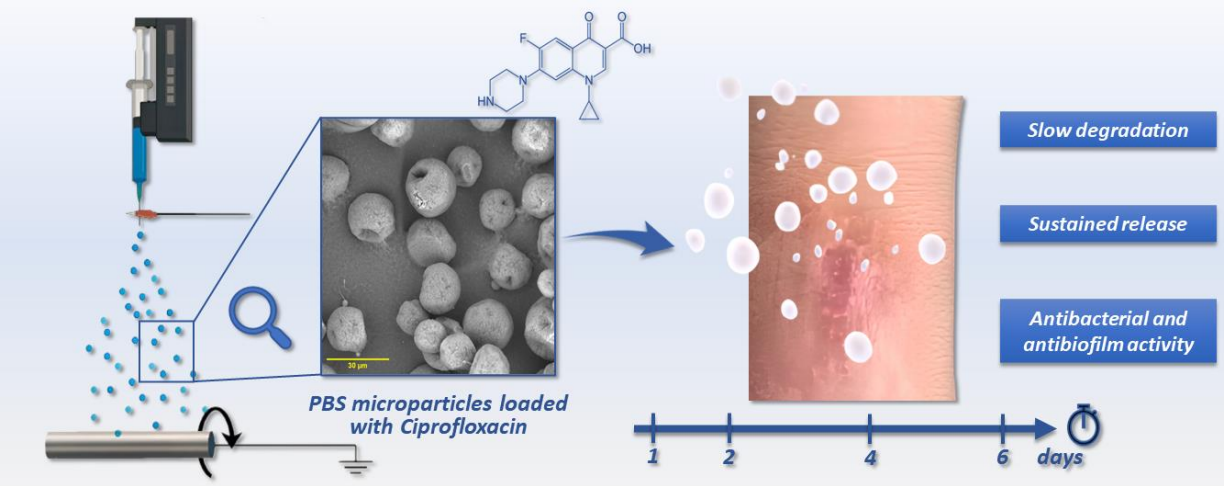

Figure 1 – Graphical abstract

In conclusion, the development of new strategies for treating antibiotic-resistant chronic infections is crucial. The use of polymeric microparticles loaded with CPX via electrospraying presents an innovative approach. These microparticles provide a sustained release of the antibiotic, effectively targeting common pathogens in chronic wounds. This study highlights the potential of locally administered slow-release broad-spectrum antibiotics encapsulated in polymeric microparticles as a promising therapeutic strategy to enhance patients' quality of life in the clinical management of chronic infections.

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Green next generation biomaterials from virtuous recovery of grape processing waste bentonite

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ABSTRACT

The waste valorisation, by conversion of discharge products into novel high value-added biomaterials, represents a virtuous strategy to contribute to the ecological transition while producing social, economic and scientific repercussions. Even more, the recycle of wastes from the local resources could maximize the impact of the circular economy idea by enhancing the territorial resources and creating new products free from additional raw materials consumption. About this, the grape processing industry is an undoubted Sicilian prestige, but it also produces abundant both organic and inorganic wastes. While grape pomace has been extensively valorised in the last years, the inorganic wastes have never been considered yet. The latter are mainly the fining agents, among which bentonite is the most common one. It is a mineral clay widely used due to low cost, abundance in nature, high clarifying power and ease of separation from the fined product by sedimentation. So far, the bentonite is just an abundant waste (100g of bentonite to fine 1hL of must/wine) then the aim of this work is to recognize it as a precious source of polyphenols to be given new life by extraction. The frozen waste black bentonite was supplied by Bono&Ditta S.p.A. Once arrived at the University of Palermo it was subjected to pulverization, sieved, divided into aliquots identified as belonging to the same lot and stored at -80°C. Samples of bentonite were subjected to green extraction by maceration (1h, 25°C, constant stirring, in the dark) choosing unconventional extraction solvents among well-known and currently used hydrophilic liquid excipients for pharmaceuticals and cosmetics. They were PEGs (PEG200, PEG400, PEG600), propylene glycol and glycerine, selected due to their high solvent power toward polyphenols and biocompatibility. The coloured liquid extracts were characterized and compared in terms of antioxidant power/scavenging activity by DPPH assay, chromatographic profile and extracted amount of some representative polyphenols by HPLC-DAD analyses, total phenolic and protein contents by Folin-Ciocalteu and Bradford assays respectively. The best extract was obtained by using PEG200 and was then further studied. It resulted stable at easily achievable storage conditions (4°C, in the dark) for at least 6 months. Furthermore, it is suitable as a novel, value-added biomaterial for biomedical and cosmetic purposes as nor skin/eye irritation neither skin sensitising potential emerged by the in vitro tests, according to OECD 439/492/442E guidelines.

Importantly, the “green soul” of this work is not just related to waste bentonite valorisation. The extraction procedure can be considered eco-friendly both in terms of employed technique and chosen

extraction solvents. This choice perfectly fit with an industrial, easily scalable and waste-to-market approach as well as with the SDGs 12, 8 and 3 of the UN agenda 2030.

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Friday October 6

Poster Session II

PASTE-LIKE SCAFFOLD FOR HYALURONIC ACID-BASED CHEMO-IMMUNOTHERAPY IN RESECTED GLIOBLASTOMA

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ABSTRACT

Glioblastoma (GBM) is the most aggressive and prevalent primary brain tumor. The current standards of care include surgical resection followed by radiotherapy and temozolomide. However, complete removal of the tumor is highly challenging and recurrences render the prognosis of GBM patients desperately poor [1,2]. We hypothesized that the inflamed environment following the tumor debulking could create the ideal conditions to locally deliver drugs aimed at enhancing the effector immune cells infiltration as well as killing the residual disseminated GBM cells. We considered a novel approach based on combining maximal safe resection with local controlled release of chemo-immunotherapeutic drugs. We combined the anticancer drugs doxorubicin (DOX) and gemcitabine (GEM) owing to their immune-related properties of inducing immunogenic cell death (ICD) [3,4] and depleting myeloid-derived suppressor cells (MDSCs), respectively. The intracavitary treatment has been finely tuned through the drug conjugation to hyaluronic acid (HA) and their consequent encapsulation within a hemostatic scaffold placed into the tumor-resected niche. Specifically, HA (100kDa) was selected for its proinflammatory adjuvant properties and selective GBM targeting; DOX and GEM have been conjugated to HA using a pH-sensitive and uncleavable linkers, respectively. HA-DOX and HA-GEM drug loading were determined by the UV-Vis method for DOX, resulting in 6.71% w/w and ^{1}H-NMR for GEM with 1.41% w/w loading. Dynamic light scattering analysis showed a size of approx. 16 nm and a negative zeta potential for both conjugates. *In vitro* investigation in 2D model of GBM murine SB28 and rat 9L cell lines showed a superior efficacy of the HA-DOX+HA-GEM conjugates (ratio 1:1 mol) compared to the free drugs, thus highlighting a superior synergistic effect; a similar trend was maintained in 3D model with higher cytotoxicity after treatment with the drug conjugates. Interestingly, we proved a higher ability of the combination of conjugates to induce ICD in SB28, by observing superior hallmarks of calreticulin exposure (2.99-fold increase) and ATP release (4.16-fold increase) compared to the free drugs alone. Finally, we developed a fit-for-purpose paste-like matrix conceived at reducing resection-induced bleeding and delivering HA-conjugates overtime. Drug release studies showed a sustained release of drugs conjugated to HA up to 30 days influenced by pH according to the linking chemistry. In conclusion, we described the potential use of a novel paste-like scaffold containing

a combination of HA-DOX and HA-GEM conjugates as an innovative approach to locally treat post-operative GBM patients, boosting the immune system to eradicate recurrent GBM.

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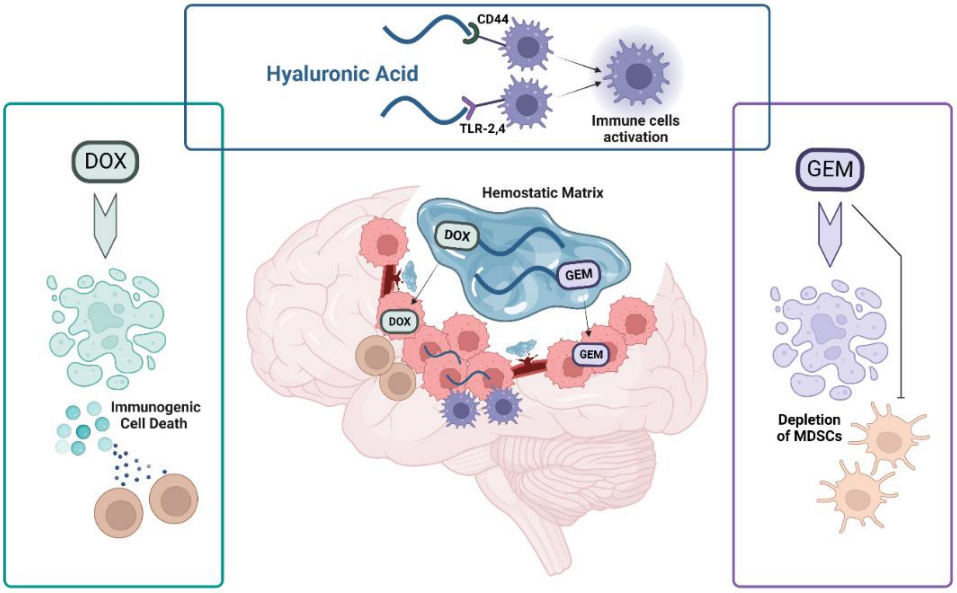

Preparation and characterization of SLNs loaded with a benzo[k,l]xanthene lignan derivative

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ABSTRACT

Benzo[k,l]xanthene lignans (BXLs) are a group of rare natural products belonging to the class of polyphenols. BXLs and other analogues show several biological properties, including antioxidant, antibacterial, anti-proliferative, DNA-binding, antifungal, anti-inflammatory, anti-angiogenic activities. LC4-BXL (Fig.1) is one derivative of these bioactive compounds that has been synthesized to improve these activities [1].

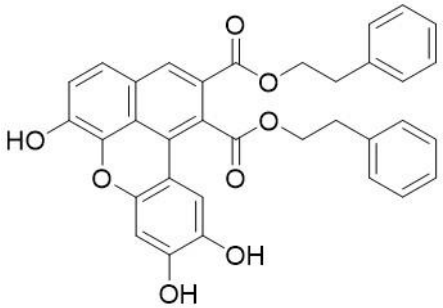

Figure 1. Molecular structure of LC4-BXL

However, the use of this molecule is limited in the pharmaceutical field due to its very low solubility in aqueous media. One of the approaches to overcome this problem is the employment of a drug-delivery system able to improve the stability and bioavailability of the compound [2]. Solid Lipid Nanoplarticles (SLNs) have been studied especially for the delivery of lipophilic compounds [3]. The use of SLNs exhibits several advantages, such as biocompatibility (since they are composed of physiological and biodegradable lipids), protection of encapsulated compounds, and drug release control [4]. In the present work, LC4-BXL has been incorporated in SLNs constituted by PrecirolATO 5, Gelucire 50/13 and Tween 80.

Size, polydispersity index, zeta potential, stability, entrapment efficiency, cellular safety, antioxidant and anti-inflammatory activity, calorimetric behaviour of SLNs loaded with LC4-BX have been assessed. The release profile has been also analysed.

SLNs have shown promising results in terms of size (about 200 nm), polydispersity index (about 0.15), zeta potential (about −16 mV) over a period of three months, and encapsulation efficiency. They have revealed antioxidant and anti-inflammatory activity in *in vitro* cellular models, and also an optimal release of LC4-BXL in the dialysis-bag experiment. Positively, the formulation does not influence the cellular viability, indicating its safety. In conclusion, based on these results, the SLNs loaded with LC4-

BXL can be proposed as a candidate for further studies. In future research, the insertion of the SLNs in a pharmaceutical vehicle for topical delivery will be assessed.

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Lipid Nanoparticles Loaded With Resveratrol And Glycyrrhetinic Acid As New Tool For Wound Healing

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ABSTRACT

Skin and mucous membranes maintain the homeostasis of the full body and are the first barriers against microbial infections. Therefore, their integrity is crucial and any lesion or injury must be quickly treated. In healthy people, several steps, such as inflammation, production of pro-oxidative species, cells proliferation and remodelling, follow each other creating a cascade process that determine the total restoration of the injured tissue. However, even a single discrepancy in these phases can delay the wound healing or irreversibly compromise the tissue. A smart strategy to promote wound healing could be the administration of natural compounds such as polyphenols and triterpenoids which are characterized by strong antioxidant and anti-inflammatory activities, antimicrobial properties and low side effects. However, the beneficial effects of these molecules are limited by their disadvantageous physico-chemical properties (e.g., low solubility in water, degradation) that compromise their bioavailability and thereby their clinical use. Based on these considerations, the aim of this work was to prepare and characterize a novel drug delivery system in form of multicomponent lipid nanoparticles (LNPs) constituted by a complex mixture of PEGylated lipid, Glyceryl monoester and Menthol able to entrap the polyphenol Resveratrol (RSV) and the triterpenoid Glycyrrhetinic Acid (GA) in order to protect them from degradation and maximize their effectiveness so as to make them useful for the wound management. Following optimization of the lipid blend composition and excipient ratios, it resulted homogeneous, with a melting range temperature of 57-61°C and containing GA (2.73 ± 0.23%w/w) and RSV (4.56 ± 0.04%w/w) in the amorphous form. The LNPs, obtained by homogenization followed by high-frequency sonication, were characterized by DLS and SEM analyses resulting almost monodispersed (PDI: 0.267 ± 0.010), with spherical shape (by SEM), nanometric size (162.86 ± 3.12nm) and suitable Z-potential (-21.40 ± 7.33mV). The quantitative analyses showed high encapsulation efficiency for both RSV and GA having a suitable DR% (96.82 ± 1.34% and 99.6 ± 1.29%, respectively) and LE% (96.82 ± 1.34% and 97.15 ± 0.19%, respectively) values. RSV release studies highlighted a sustained and controlled pattern of discharge to different chemical environments simulating the wound conditions. Moreover, LNPs showed significant scavenger properties evaluated by the DPPH assay. Last, the biological evaluations (scratch assay) highlighted an enhanced fibroblasts proliferation and migration at extremely low doses (LNPs 22 μg/mL corresponding to RSV 5 μM).

Furthermore, a promising antibiofilm effect against *Staphilococcus aureus* was observed in a dose-dependent manner. In conclusion, these novel multicomponent LNPs could represent a next generation carrier constituting a promising tool for wound healing purposes.

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Mannose receptor-targeted glycopolymers for the treatment of inflammatory diseases

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ABSTRACT

INTRODUCTION

The mannose receptor (MR) is a glycoprotein expressed by macrophages and immature dendritic cells, potentially involved in the development of inflammatory diseases.1 A possible role of MR in inflammation involves the macrophage MR-mediated endocytosis of the enzyme myeloperoxidase (MPO), released by neutrophils at inflammation site, which induces the production of reactive oxygen species (ROS) and pro-inflammatory cytokines2-3. Novel sulfo-galactosylated glycopolymers (SG) here discussed selectively block MR endocytic activity thus ideally preventing the internalization of MPO by macrophages and its pro-inflammatory effects. Galactosylated glycopolymers (GG), not binding MR, were used as negative control.

METHODS

SG and GG were generated by fast Reversible Addition-Fragmentation chain Transfer (RAFT) polymerization and characterized by 1H NMR and GPC.

Bone marrow cells were cultured in M-CSF containing medium to obtain naïve macrophages, then polarized in M1 and M2 phenotypes with 50 ng/mL IFN-γ and 10 ng/mL IL-4, respectively.

For ROS production, M1 and M2 macrophages were pre-incubated for 45 min with a 50 μM 2',7'-dichlorofluorescin diacetate (DCFH-DA) solution in HBSS. Then, the solution was replaced with 600 μM SG or GG solution in HBSS. After 1 h incubation, 25 μg/mL MPO solution in HBBS was added. The fluorescence intensity was measured every 15 min over a 4 h period. Control cells were incubated with HBSS or MPO only.

For cytokines detection, M1 and M2 macrophages were incubated for 45 min 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 specific wells. Cells were incubated for a further 6 h. Afterwards, supernatants were collected and tested with ELISA kits for TNF-α and IL-1β detection.

RESULTS AND DISCUSSION

Via RAFT polymerization we obtained well-defined polymers (SG, Mn 47763, PDI 1.28; GG, Mn 56300, PDI 1.35). Through ROS kinetic assay, we observed a decrease of ROS generation of 13.5% and 25% by M1 and M2 macrophages, respectively, when pre-incubated with SG followed by addition of MPO as compared to cells incubated with MPO alone (*Figure 1A,B*).

Concerning pro-inflammatory cytokines production, while no difference in the TNF-α release was detected (data not shown), IL-1β levels in the samples treated with SG+MPO decreased by 31% in both cell lines when compared to the sample only stimulated with MPO (*Figure 1C,D*).

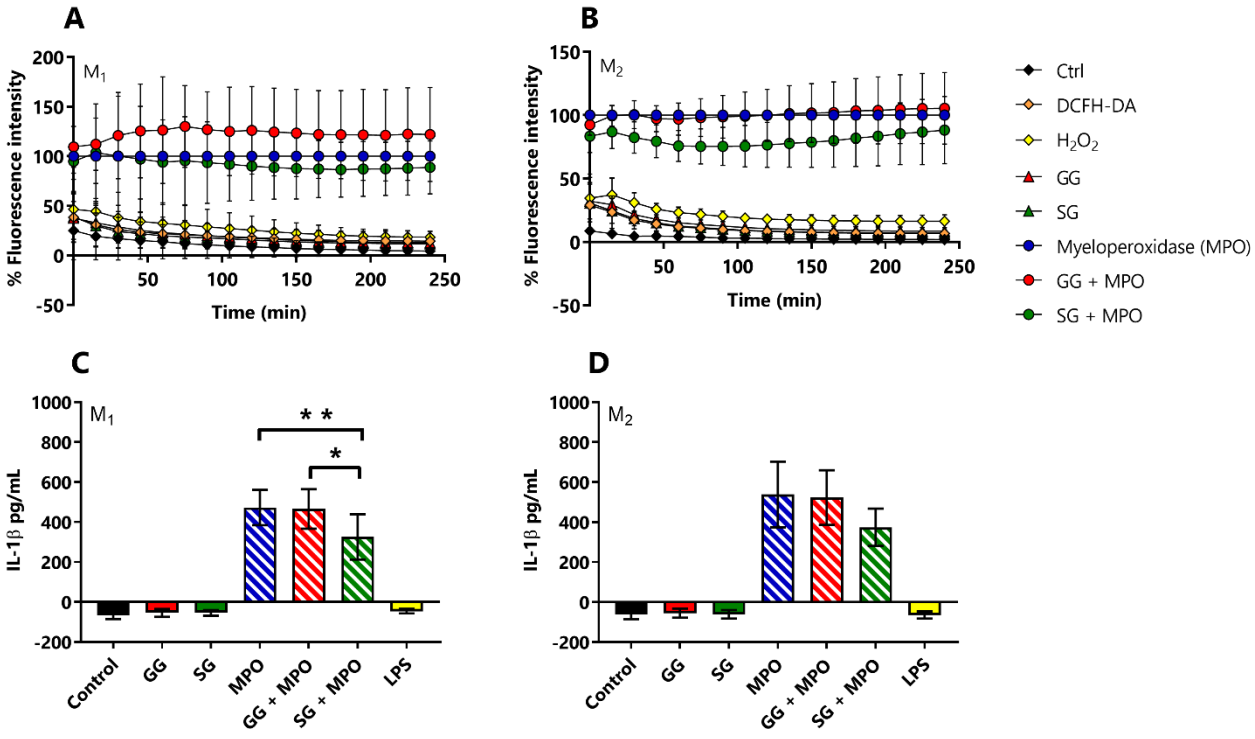

*Figure 1. ROS production and IL-1β quantitation on M_1 (A,C) and M_2 (B,D) macrophages treated with SG/GG in presence or absence of MPO. P<0.05; **, P<0.01.*

Importantly, pre-incubation with GG followed by MPO addition did not show any anti-inflammatory effect, supporting the specificity of the mechanism.

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Double crosslinked polysaccharide/polyaminoacid printable hydrogels for bone regeneration

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ABSTRACT

Bone defects are often one of the main causes of morbidity and disability worldwide[1]. In particular, alveolar and maxillofacial bone defects caused by trauma, extraction or inflammation processes, are difficult to treat with traditional procedures[2,3]. For these reasons, innovative approaches of bone regenerative medicine represent a promising strategy to overcome the problem. The development of injectable and printable hydrogels, and specifically polysaccharide-based ones, received widespread interest and demonstrated great potential for this purpose[4–7]. In this work, we developed printable double crosslinked hydrogels based on oxidized xanthan gum and methacrylate polyaspartylhydrazide containing hydroxyapatite nanoparticles (Figure 1). In particular, we exploited the hydrazone bond formation between a polysaccharide, an oxidized xanthan gum (XGox), and a polyaminoacid, a methacrylated polyaspartylhydrazide (PAHy-MA). Thanks to the presence of this kind of bond, the hydrogels showed pseudoplastic behaviour and good recovery of viscoelastic properties over time: an important aspect for printable hydrogels. The presence of methacrylic groups on PAHy-MA, on the other hand, made possible to photo crosslink the scaffold during the printing process improving its viscoelastic properties and stability in physiological fluids and, also, maintaining the shape. Moreover, the use of a PAHy-MA, thanks to PAHy properties[8], but also the absence of biomaterial derived from animal sources, represent a great advantage for the application in bone regenerative medicine. The obtained systems have been characterized in order to evaluate their physicochemical, rheological and biological properties. Therefore, the system could be a potential candidate in the treatment of bone lesions as an injectable hydrogel, allowing to fill irregular defects like that of alveolar bone, and as a 3D bioprinting bioink.

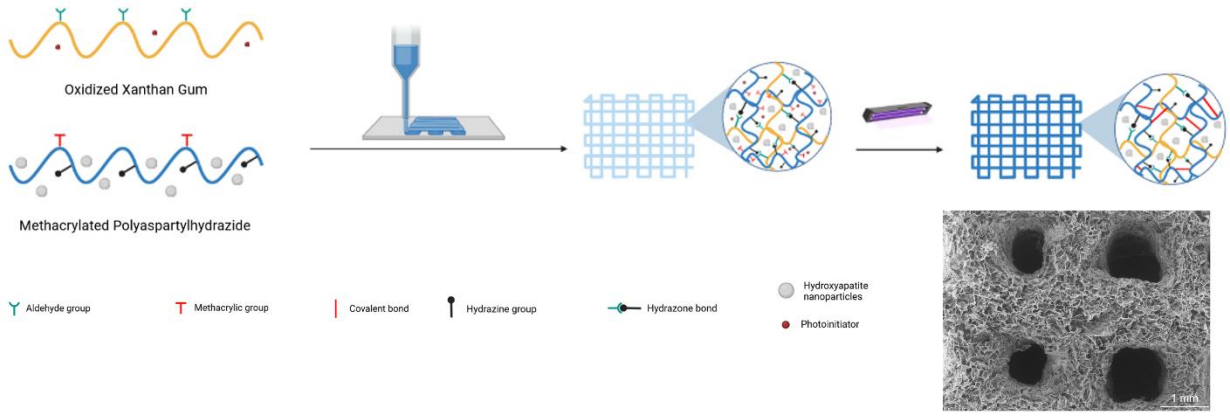

Figure 1. Schematic representation of the development of XGox/PAHy-MA based 3D printed scaffolds.

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Dendritic/gellan gum hybrid hydrogels with antibacterial broad-spectrum activity

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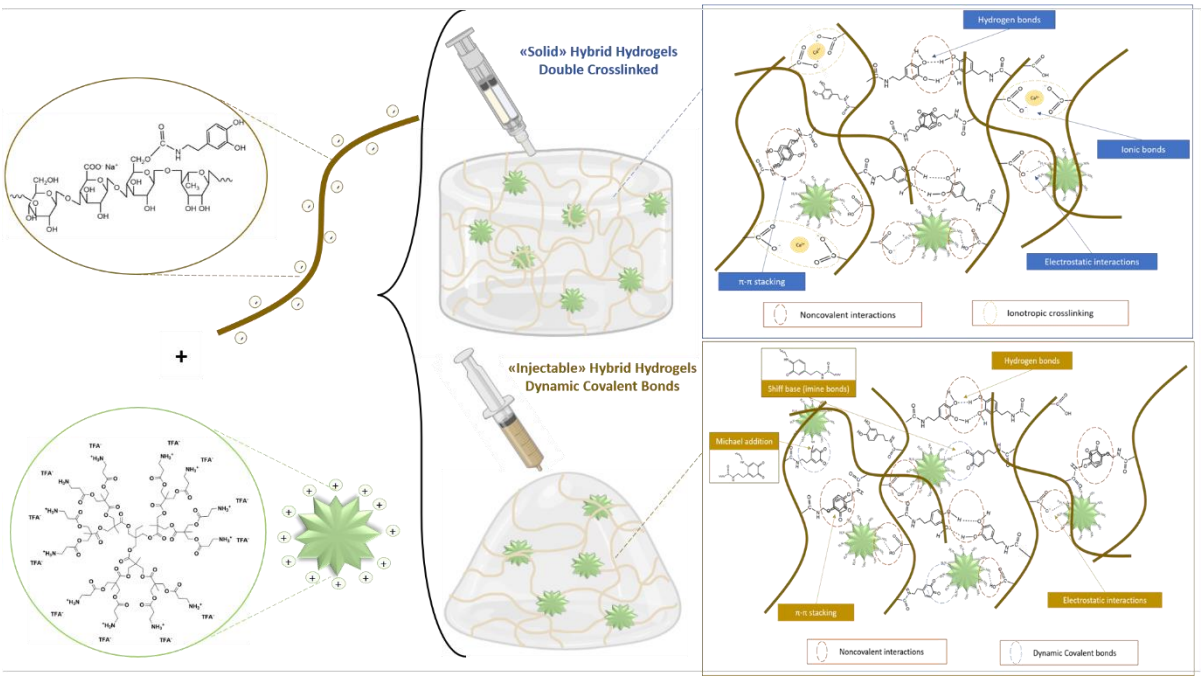
ABSTRACT

In recent years, as pathogenic bacteria have started to develop resistance to common antibiotics, treating infections has become a difficult task (1). In the event of a skin injury, infection and pro-inflammatory factors can lead to chronic inflammatory reactions that can significantly delay wound healing (2). It is imperative that more advanced wound dressing solutions are developed and deployed to efficiently eradicate infections and thereof accelerate the recovery of the traumatized tissue (3,4). This study develops hybrid hydrogels based on dopamine-functionalized Gellan Gum (GG-DA) and TMP-G2-alanine cationic dendrimer with broad-spectrum antibacterial activity (5). The electrostatic interaction between the anionic charges of GG-DA and the cationic fractions of TMP-G2-alanine determines the gelation of the hybrid system. The hydrogels thus obtained can be doubly crosslinked with $CaCl_2$, obtaining hybrid hydrogels called "solids". Furthermore, by increasing the pH of the GG-DA precursor solution, hydrogels with different rheological properties (compared to the ionically crosslinked one) can be obtained due to the formation of additional bonds such as the Shiff base and Michael's addition between o-quinone groups of dopamine oxidized with amine groups of the dendrimer. These hydrogels could be extruded from the needle of a syringe, so they were called "injectables". Both hydrogels produced have good swelling profiles and biodegradable properties. The injectable hydrogels also show good shear-thinning and self-healing properties. The hybrid hydrogels showed good cytocompatibility, up to 100% viability of three different cell lines, human dermal fibroblasts (HDF), human epidermal keratinocytes (HaCaT), and mouse monocyte cells (RAW 264.7). The hydrogels show an adhesive behavior to various substrates, among which porcine skin. These hydrogels possess highly interconnected porous structures and exhibit also live cell-adhesion properties on their surface. At the same time, the dendrimer served to crosslink the hydrogels and endow them with excellent broad-spectrum antimicrobial activity within four hours, as evaluated using two representative bacterial strains, S. aureus 2569 and E. coli 178. This study demonstrates

that using the same GG-DA/TMP-G2-alanine ratios, it is possible to produce multifunctional hybrid hydrogels with tunable properties and great potential for wound dressing applications.



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Design of insulin granule-loaded microPlates for the treatment of type-1 diabetes

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ABSTRACT

Type-1 diabetes (T1DM) is a chronic endocrine and metabolic disorder that affects approximately 30 million people worldwide, which is characterized by the destruction of β-cells through autoimmunity [1]. The standard treatment involves subcutaneous insulin injections and monitoring of blood glucose levels (BGLs), but this can be challenging for patients and increase the risk of hypoglycemia resulting in severe complications [2].

In this context, innovative controlled delivery systems have shown promising potential in enhancing diabetes treatment by improving drug stability, overcoming biological barriers for increased drug availability, and acting as automated systems to mimic natural insulin delivery and reduce the risk of hypoglycemia.

Herein, we developed structured polymeric particles called insulin-microPlates (INS-uPL), composed of a biodegradable and biocompatible poly(lactic-*co*-glycolic) acid (PLGA) matrix loaded with insulin.

Inspired by the microcrystalline formulations commonly used in the pharmaceutical industry, we employed a crystallization process to prepare insulin granules. Briefly, zinc acetate (ZnAc) and/or zinc chloride ($ZnCl_2$) (0.05 M), trisodium citrate (0.05 M) and acetone (15% v/v) were added to a solution of insulin (5 mg/mL) dissolved in acidified water (HCl 10 mM, pH = 2.5), poured by a microfluidic system (5 μL/sec) [3]. Considering that crystal size could affect insulin dissolution and diffusion, we produced different crystals by varying ratios between zinc salts (chloride and acetate), ranging from 10 to 100% of $ZnCl_2$/ZnAc, to control crystal size.

Dimensional analysis of the insulin granules was performed by dynamic light scattering (DLS), which showed an average size ranging from 259 ± 2.36 nm (ZnAc 100%) to 1073 ± 167 nm ($ZnCl_2$ 100%). The granules displayed a negative surface charge of -20 ± 4.92 mV. To protect the crystals from rapid dissolution in biological fluids, we uniformly dispersed insulin granules within the polymeric matrix of microPlates. The INS-μPL were obtained through a multi-step replica molding process, which involved a top-down fabrication approach.

The morphology of the hierarchical particles was analysed using scanning electron microscopy (SEM) (Figure 1), confirming the squared shape of INS-uPL of 20 x 20 µm. Multisizer Coulter Counter further assessed the dimension of the produced particles, indicating a peak size ranging between 15 and 30 µm, with the highest and resolute peak at 20 µm. Additionally, we determined the encapsulation efficiency (EE%) of insulin crystal (ranging from 66 to 91± 19.2%) and the loading efficiency (LE%) of INS-uPL (ranging from 3.64 to 5.07 ± 1.24%) using ultra-performance liquid chromatography-tandem mass spectroscopy (UPLC-MS) analysis. Eventually, based on these outcomes, further studies will be carried out, specifically, to evaluate the release kinetics of INS-uPL by tuning INS crystal size and polymeric matrix concentration.

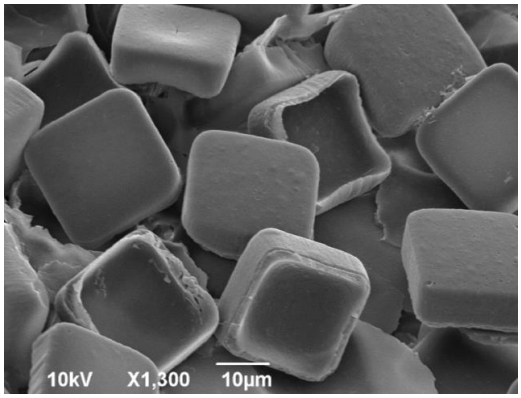

Fig.1: Representative SEM image of INS-µPL.

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Schiff-base based hydrogels for the generation of 3D printed liver assembloids

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ABSTRACT

Assembloids are 3D structures formed from the fusion and functional integration of multiple cell type, and, most important, they mimic the complex cellular interactions from which organs arise in the body[1]. In particular, liver assembloids are crucial to establish as *in-vitro* models that can recapitulate hepatic diseases/injuries, such as Ischemia/Reperfusion Injury (IRI), providing a disease model usable for drug screening to ultimately pave the way for new therapies. In the last few years, natural polymer-based hydrogels became essential to generate assembloids which resemble their environment within the tissues. However, these hydrogels can result in too fast reabsorption and their application can be limited by poor mechanical properties. In addition, permanently cross-linked hydrogels are not appropriate in cell delivery, representing an obstacle to cell growth. On the other hand, polymeric networks with reversible linkages can be used to allow cells interaction and remodeling with the ECM-like structures with consequent proliferation and migration.

The Schiff base is used in click chemistry to generate dynamic linkages and reacts in mild and physiological conditions allowing to obtain cell loaded hydrogels. Moreover, this reaction, due to the shear thinning and self-healing properties, has excellent prospects in 3D bioprinting[2].

Considering the structural and important role in biological mechanisms, hyaluronic acid (HA) was selected and chemical modifications were performed to introduce aldehyde (HA-Ald) and amino nucleophilic groups (HA-DETA) to the polysaccharide backbone. Therefore, in this study, HA-DETA/HA-Ald hydrogels were fabricated in order to generate liver assembloid 3D platforms. Hydrogels showed suitable rheological properties, tunable mechanical properties and chemical stabilities, a short healing time, and a quick gelation time useful for homogeneous mix of encapsulated cells. Biomaterials with these features are able to quickly self-repair after injection or extrusion providing mechanical support or protection for the loaded cells during the 3D process.

Preliminary studies with different polymer concentration, molar ratio and cell density were performed to set best cell embedding conditions and 3D printability. Subsequently, cells viability was tested in hydrogels over 21 days. The dynamic crosslinking resulted beneficial to cell extension and growth,

as all the different types of encapsulated cells were metabolically active at all the time-points analyzed. In addition, the hydrogel-based assembloids could be analyzed for molecular and cellular re-organization, as we have been able to isolate RNA, proteins, and we could also embed the polymers in paraffin to perform hematoxylin/eosin stainings. All these aspects are extremely important to fully understand the quality of the generated liver assembloids.

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Self-assembled hybrid nanoparticles: a formulative study for active targeting strategy

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ABSTRACT

Polymer and lipid-based nanoparticles (NPs) represent two dominant classes of nanocarriers capable of efficiently encapsulating and delivering a variety of drug classes. Given the clinical success of biodegradable and biocompatible nanocarriers, we hypothesized that a lipid-polymer hybrid NP may be developed taking advantage of the unique strengths of polymer and lipid-based NPs [1].

Hyaluronic acid (HA) is an anionic glycosaminoglycan that has received particular attention for its interesting targeting ability being its receptor, CD44, overexpressed on different solid tumors [2]. We previously prepared conjugates ($HA_{4.8}$-DPPE) obtained by linking HA at the low molecular weight (4.8 kDa) to an aminated phospholipid (1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine, DPPE) by reductive amination [3]. In this study, we used acid-terminated poly-(lactic-*co*-glycolic) acid (PLGA) as a model hydrophobic polymer to form the polymeric core of the NPs surrounded by HA. The association of HA on the surface of NPs occurred through the nanoprecipitation of PLGA into an aqueous solution of $HA_{4.8}$-DPPE conjugate. PLGA-HA-DPPE NPs were self-assembled making the DPPE inserted into the polymer matrix and exposing the HA on the surface. NPs were characterized by a size below 140 nm and a negative zeta potential around -60 mV. As model drug, we focused on pentamidine free base (PTM-B), a diamidine compound approved as an antiprotozoaric drug and recently repurposed for its potential anticancer activity or in the treatment of muscular dystrophies. To examine the encapsulation efficiency of PLGA-HA-DPPE NPs, we compared them to our previous formulation of PTM-B-loaded PLGA NPs [4]. The amount of loaded drug was higher than that of PTM-B-PLGA NPs. Moreover, by increasing the amount of PTM-B in the formulation, the size decreased up to 90 nm, still maintaining a negative zeta potential of -45 mV. Probably, the presence of HA on the surface of NPs enhanced the ionic interactions between the negatively charged NP and the positively charged PTM-B. Furthermore, to increase the long-term storage stability of hybrid NPs, we exploited the cryoprotection activity of HA associated to the surface of PLGA NPs. We freeze-dried hybrid NPs obtaining a workable cake, able to be resuspended after the lyophilization process. The NPs retained their size after resuspension and their spherical shape. Considering the targeting effect of HA, these results pave the way for the use of HA-DPPE conjugate to formulate actively targeted hybrid nanocarriers.

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Microfluidic production of pDNA-loaded lipid NPs: optimization and *in vitro* efficacy

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ABSTRACT

Nanoparticle-based gene therapy has been increasingly growing in recent years for both genetic and non-genetic diseases, especially given the success of the lipid nanoparticle (LNP) based COVID-19 vaccine. Among all known methods, microfluidic technology is the new paradigm to produce LNPs, but most aspects of this novel technique need to be more deeply investigated.

In this study, fluorescent cationic LNP formulations were optimized to load GFP coding plasmid DNA using the microfluidic technique. Technique parameters such as chip type, Total Flow Rate (10 mL/min), Flow Rate Ratio (1:1), lipid concentration (5 mg/mL) solvent (Methanol), and structural components of the LNPs (DPPC and Cholesterol) were held constant. The other formulation-related parameters were optimized including: the amount of cationic and fluorescent lipids, Dotap and DOPE-Rhod respectively, and the composition of the aqueous phase (water or buffer). All formulations were characterized for size, polydispersity, surface charge, morphology, fluorescent intensity, stability to storage conditions, DNA loading capacity. After the optimization of the empty formulation of LNPs, the resulting formulations were tested for their ability to either bind (post-addition) or encapsulate (DNA present during formation) plasmid DNA. For the more classical post-addition method, LNPs were first formulated empty, and a solution of plasmid was added to the LNP suspension to arrive at a final plasmid concentration of 5, 10, or 15 μg/mL. This method showed critical limitations, as the LNPs showed aggregation with increasing concentrations of pDNA. On the contrary, by adding the plasmid into the aqueous phase it was possible to bind larger amounts of pDNA up to 100 μg/mL even with a wider range of N:Ps ranging 25:1 - 1:1, with almost 100% loading efficiency. The formulations with the largest amount of DNA still resulted in liposomes of small size, that were stable upon storage at 4°C, and retained the loaded DNA over several weeks.

The importance of the cationic lipid was also evaluated with both loading methods. With the classical method, in the absence of cationic lipid, LNPs loaded < 2% pDNA as expected due to lack of electrostatic interactions. Surprisingly, even without the cationic lipid, when the pDNA was solubilized in the aqueous phase during the formulation, almost 40% of the DNA was loaded in the LNPs. These formulations represent a great advantage in the field of gene therapy, as they can facilitate the protection and possible delivery of therapeutic plasmids with a reduced amount of cationic lipids, which are often toxic, with increased DNA content. The toxicity and transfection efficacy of the most promising formulations are now under investigation using *in vitro* models to assess the optimal balance between reduced cationic lipid and transfection efficiency.

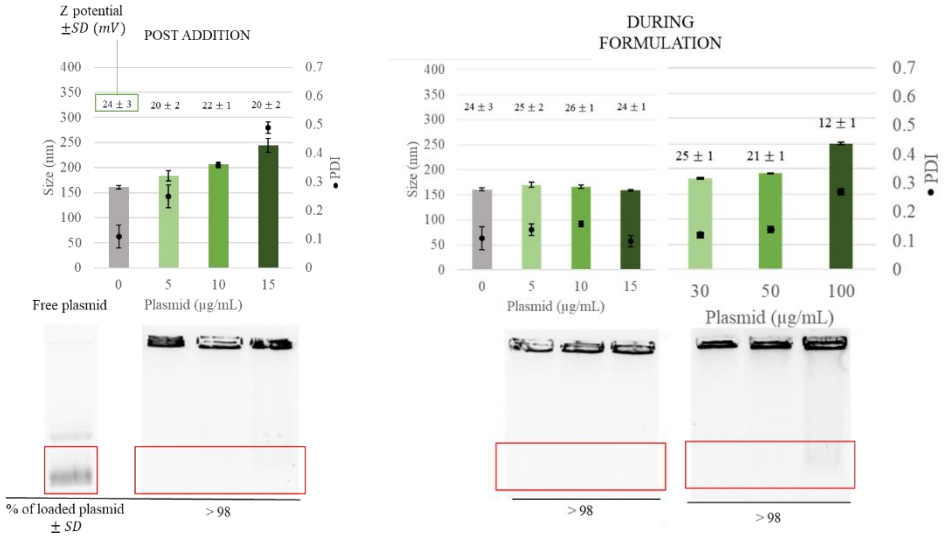

Fig 1: Chemico-physical characteristics and loading efficiency with the plasmid added post or during formulation.

Ketorolac loaded PLGA coating of AZ31 alloy: development and characterization

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ABSTRACT

AZ31 is a biodegradable magnesium alloy and it has potential as support for favouring bones repair. Despite positive aspects, it is characterized by rapid degradation in physiological conditions [1]. PLGA could slow down the degradation process [2] and control the release kinetic of active pharmaceutical ingredients. In this work, ketorolac tromethamine (KT) loaded PLGA was used for AZ31 coating.

AZ31 disks were activated in a sodium hydroxide (NaOH) solution and successively heated to stabilize the activated layer. Then, samples were coated with PLGA containing 5% w/w KT using the solvent casting technique. Raman spectroscopy, SEM and DSC were used to characterize the coated samples. *In vitro* release profile and blank PLGA mass loss were determined. *In vitro* cytotoxicity studies were performed to evaluate KT [3, 4] and AZ31 coated samples effects on BSCL138 fibroblasts and primary human osteoblasts.

Raman spectra revealed the presence of newly formed hydroxyl groups on the sample surface after activation, evidenced by the bands at 3664 cm^{-1} and 3690 cm^{-1} (Figure 1) [5]. Additionally, the Raman technique provided an estimation of the PLGA coating thickness, which was approximately 300 µm. SEM photomicrographs allowed to distinguish between AZ31 and polymeric coating. Besides, a stronger adhesion of PLGA to activated AZ31 was observed compared to non-activated alloy. The glass transition temperature of PLGA was lowered of ~ 14 °C in respect of raw PLGA due to KT plasticizing effect. Since only one face was exposed to solvent, the release rate of AZ31 coated samples was slower (80% after 15 days) than KT loaded PLGA film and no burst release was observed. Blank PLGA mass loss showed a sigmoidal trend reaching 95% after 45 days. Importantly, KT cytotoxicity on osteoblasts and AZ31 cytotoxicity on both cell types were significantly reduced in PLGA coated samples.

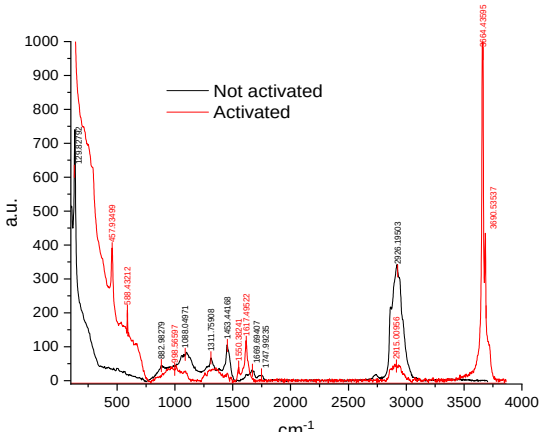

Figure 1. Raman spectra of activated (red) and non-activated (black) AZ31 samples.

PLGA coating was successful in slowing down AZ31 degradation, preserving the alloy mechanical properties, and in improving its cytocompatibility. Moreover, KT release kinetics was compatible with its use for pain relief in bone fracture.

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Synthesis and electrospinning of PGS-cinnamoyl polymerfor manufacturing elastomeric scaffolds

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ABSTRACT

In the design of synthetic biodegradable vascular grafts, the structural biomaterial is chosen to mimic blood vessels' ability to expand and recoil to their original shape in response to blood pressure[1]. Hence, to produce synthetic vascular grafts, biomaterials with appropriate elastic properties need to be developed. Polyglycerol sebacate (PGS) is a biodegradable, biocompatible elastomeric material[2], but with a main drawback: its synthesis is extremely energy and time consuming[3], discouraging its use in tissue engineering and drug delivery, despite its high-potential. In this work, alternative synthesis of PGS was investigated combining and optimizing microwave synthesis[4] and UV curing[5], with the goal to completely perform PGS elastomer synthesis within 72 hours and in a standardized and reproducible way. Pre-PGS was synthetised heating equimolar amounts of sebacic acid and glycerol in a closed vial at 230°C for 15 minutes, using a Biotage Initiator 2.0 (400W) microwave reactor. FTIR analysis confirmed pre-polymerisation and a degree of esterification of 79.1±0.8% was reached. Gel Permeation Chromatography and Mass Spectroscopy analyses were performed to determine pre-PGS molecular weight, that ranged between 0.25 and 2.17 kDa with a PDI close to 1 for each molecular species. Incorporation of pendant UV-reactive cinnamate groups was obtained reacting pre-PGS with 50% or 100% mol/mol cinnamoyl chloride, DMAP and TEA. H-NMR confirmed the synthesis of pre-PGS-Cin, that was further blended with PLGA to perform electrospinning and obtain fibrous mats. UV-curing was achieved by irradiating the fibrous mats with a wide spectrum UV-A lamp for 12 to 48 hours. Morphological, physico-chemical and mechanical characterization of PGS-Cin/PLGA blend electrospun mats is currently ongoing.

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Nano into Micro (NiM) formulation based on lipid-polymer hybrid nanoparticles for pulmonary siRNA delivery

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ABSTRACT

Inhalation gene delivery by nanomedicine is a recent breakthrough therapy for the treatment of several lung diseases, thanks to the ability to complex, protect and transport the genetic material across lung barriers to the cellular target. (1) In this regard, lipid-polymer hybrid nanoparticles (LPHNPs) have recently been proposed for their unique advantages arising from polymeric nanoparticles and liposomes for efficient siRNA encapsulation and delivery. (2)

Along these lines, the aim of this work was the development of a novel formulation based on LPHNPs for pulmonary delivery of siRNA. Specifically, our LPHNPs were made by a polymeric core of a fluorescent cationic graft polyaspartamide/poly(lactic-co-glycolic) acid conjugate (PHEA-bAPAE-g-DY676-g-PLGA), and a lipid shell of a mixture between 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), which is the major component of pulmonary surfactant, and 1,2-distearoyl-sn-glycero-phosphoethanolamine-N-(polyethyleneglycol)$_{2000}$ (DSPE-PEG$_{2000}$), a pegylated phospholipid which could confer stealth and mucodiffusive properties to the resulting system. (3)

PHEA-bAPAE-g-DY676-g-PLGA was obtained by chemical conjugation of 1,2-Bis(3-aminopropylamino)ethane (bAPAE), DY676 and PLGA on the PHEA backbone, which confer, respectively, the ability to complex the gene material via electrostatic interactions, fluorescence, and amphiphilic properties to obtain polymeric nanoparticles.

LPHNPs-loaded siRNA (LPHNPs@siGFP) were prepared by emulsion/solvent diffusion, with colloidal size, positive ζ potential, a core-shell morphology and consisting of 30 wt% phospholipids. They are able to incorporate successfully siGFP and interact slightly with mucin. LPHNPs are well internalized into lung cancer cells and have knockdown capabilities.

To overcome the aerodynamic limitations of the nanocarrier for inhalation, an inhalable powder composed of LPHNPs@siGFP and trehalose was obtained by using the NiM strategy and realized by spray-drying. (4) Spherical NiM particles with suitable dimensions and excellent aerosolization properties for optimal lung deposition were produced.

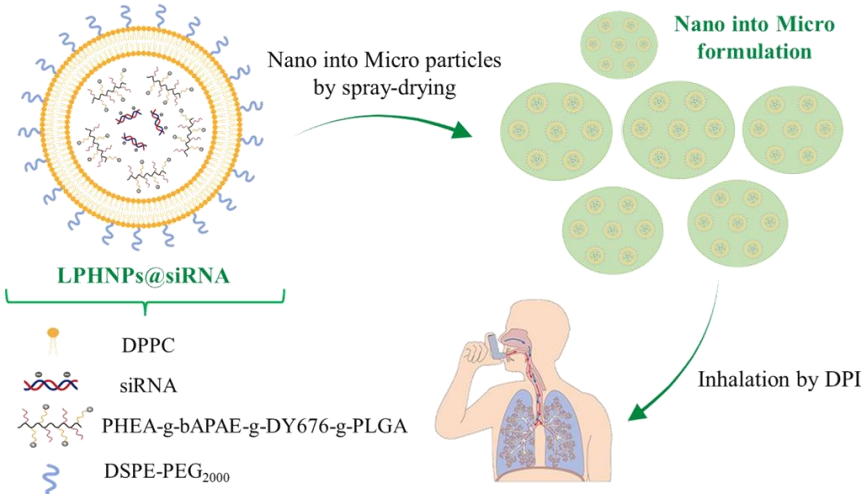

Fig. 1. Schematic representation of the work.

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Safe and efficient mRNA vaccine delivery using lipid nanoparticles

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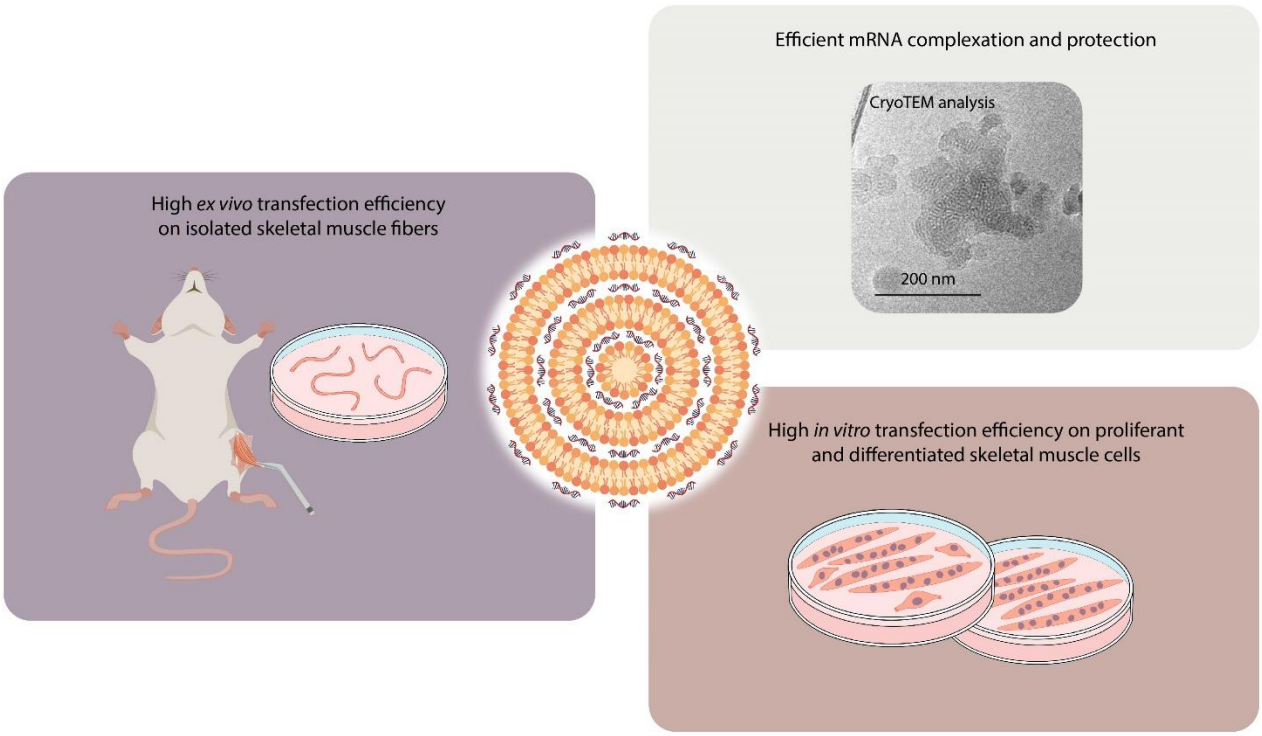
ABSTRACT

The recent success of mRNA-based COVID-19 vaccines has impressively highlighted the therapeutic potential of nucleic acid-based therapeutics. However, delivery of the nucleic acid payload to the target cells is still a major challenge within the development of mRNA-based therapeutics [1]. Over the last years, various delivery vectors have been extensively investigated for nucleic acid delivery. Among them, lipid nanoparticles (LNPs) have gained great interest from groundbreaking research to cutting-edge applications with the commercially approved Onpattro® and mRNA-vaccines [2,3].

The purpose of the present study was to develop mRNA-lipid nanoparticles for modulating protein expression in skeletal muscle cells as proof-of-concept for vaccine applications. In fact, transfecting muscle cells is a coveted milestone to ensure the production of the viral antigens and trigger the specific immune response. As proof-of-concept to modulate protein expression in skeletal muscle cells, eGFP fluorescent protein was selected as reporter gene. The present investigations demonstrated the high efficiency of our recently patented LNP (FR2112931) to associate mRNA [4]. All complexes were monodispersed and characterized by a hydrodynamic diameter around 200 nm. The most suitable nanosystems were then tested *in vitro* on C2C12 murine skeletal muscle myoblasts and myotubes. Data demonstrated a high transfection efficiency with up to 90 % of myoblasts and 80 % of myotubes expressing eGFP without any overt signs of toxicity on both cell types. Finally, nanosystems were tested *ex vivo* on muscle fibers explanted from healthy mice. Results demonstrated that nanosystems were accumulated at the membrane level and a high eGFP expression was observed following 24 hours, highlighting the high efficiency of our nanosystems to transfect skeletal muscle cells.

Overall, the present study demonstrates the suitability of our LNP to deliver mRNA in skeletal muscle cells, opening the range of possibilities for vaccine applications. Further investigations will be devoted to study antigens expression *in vivo*.



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3D printing in the pharmaceutical field: A tool to personalize medicines

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ABSTRACT

Nowadays, 3D printing is revealing its potential for pharmaceutical applications thank to its flexibility in terms of applications, materials, and available printing technologies.

Our aim, here, is to present our different approaches on the utilization of 3D printing technologies to produce pharmaceutical forms and medical devices (*e.g.*, patches, intravaginal rings), manufacturing devices (*i.e.*, microfluidics and solution blow spinning) and analytical devices (*i.e.*, vertical diffusion cells). The main advantage in the use of the 3D printing technology is the possibility to personalize the final product based on patient or researcher needs.

As medical devices, we were able to produce antifungal 3D printed intravaginal rings using thermoplastic polyurethane and ethyl vinyl acetate (EVA) loaded with clotrimazole, and bifonazole [1]. These rings showed a sustained release and an efficient in vitro activity against *C. Albicans*, the pathogen generating vulvovaginal candidiasis. Moreover, with direct powder extrusion (DPE) 3D printing, Polyhydroxybutyrate (PHB) was employed as biodegradable polymer to produce prolonged drug release devices [2], meanwhile EVA was used to produce transdermal patches.

As manufacturing tools, we developed 3D printed microfluidic chips using polypropylene (PP). Taking advantage from these devices, we formulated a wide library of innovative nanocarriers using lipids, polymers, proteins, and polysaccharides in a controllable, tunable, and scalable way [3]. Moreover, we have been able to 3D print a solution blow spinning device that allows to produce fibers without the application of an electric field starting from polymer's solutions in water.

Finally, as analytical device we built a 3D printed vertical diffusion cell that can be efficiently used instead of glass ones to evaluate both drug release and permeation [4].

3D printing has opened a new era in the pharmaceutical field. We strongly believe that in the close future, 3D printing will revolutionize the approaches to produce personalized medicines and pharmaceutical relevant manufacturing and analytical devices.

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Film forming spray containing colistin loaded albumin nanoparticles to tackle antibiotic resistance

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ABSTRACT

Antimicrobial resistance is a major health emergency. Multidrug-resistant (MDR) Gram-negative bacteria (GNB) such *Enterobacteria*, *Acinetobacter baumannii* and *Pseudomonas aeruginosa* are responsible for hospital infections (e.g. septicaemia, pneumonia, skin and soft tissue infections SSTIs). In particular, SSTIs from MDR-GNB are occurring frequently in the clinics, especially in patients with immunodeficiencies, diabetes and burns. Colistin (Col), despite its poor tissue distribution and toxicity, is increasingly used as a 'last-line' therapy for the treatment of these infections for which no other options are available. The emergence and spread of resistance to Col further reduces the therapeutic possibilities and makes it necessary to develop new formulations that are more effective and able to overcome the resistance mechanisms established by the bacteria.

Here, we developed a spray formulation for the topical delivery of Col by loading the drug in chitosan-coated human albumin nanoparticles (Col/haNPs) that we have previously developed and patented[1,2].

Col/haNPs are cytocompatible and show high antimicrobial and antibiofilm efficacy against MDR strains of *A. baumannii* and *K. pneuomaniae KPC* strains, both susceptible and resistant to Col. The composition of the formulation has been optimized to enhance the sprayability and spray coverage area. The inclusion of Col/haNPs in the spray does not significantly influence the chemical-physical parameters, the rheological behaviour of film-forming solutions or the spray angle of the formulation. To assess whether the resistance mechanism influences the response to Col/haNPs, selected strains were sequenced and evaluated for the presence of efflux pumps, mcr-1 gene and possible loss/modification of LPS. All MDR GNBs on which NPs reduced the minimum inhibitory concentration (MIC) of Col, were mcr-1 negative and did not lack LPS. High impact single nucleotide polymorphism was identified in the pmrA and galU genes involved in LPS modification. Furthermore, phenotypic tests suggest a role of efflux pumps in the resistance of the tested strains. Preliminary data obtained using an *ex vivo* human skin model of infection with *A. baumannii* resistant to Col indicate that the Col/haNPs spray formulation is able to significantly reduce the number of bacteria.

Our results show a new antibiotic loaded nanotool able to bypass drug resistance and being effective on Col-resistant bacteria. The administration through a film-forming spray formulation allows for easily application, controlled dosing and sustained drug release by reducing the dose and number of administrations required.

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Arginine-rich peptidomimetic antibiotics-polymer conjugate for combined treatment of nosocomial biofilms

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ABSTRACT

The exacerbation of antibiotic resistance is a major threat to healthcare worldwide being the result of manifold stubborn factors, including improper antibiotic therapies and insufficient advances in the discovery of new active molecules. [1] This framework points out the hardship in dealing with tenacious pathogens, making the treatment of severe infections still challenging. Combined therapy with penicillins and aminoglycosides has been proved beneficial to address many persistent bacterial infections with possible synergistic effects. However, the different pharmacokinetic profiles of these two antibiotic classes may not guarantee a concerted spatio-temporal delivery at the site of action, decreasing the efficacy of this combination and promoting resistance. Herein, we designed a polymer-antibiotic conjugate where ampicillin and gentamicin were grafted into a biocompatible polyamino acidic and arginine-rich backbone aiming to supply a unique smart solution to circumvent biodistribution and half-life discrepancies of ampicillin/gentamicin therapeutic association (Figure 1). To do this, we adopted a synthetic procedure with a proven wide versatility that offers a simple and cost-effective approach to design synthetic derivatives with protein-like amino acidic structures. [3-4] The antibiotic molecules were covalently bonded as side pendants to the hydrophilic PAA backbone, obtaining the polymer−antibiotic conjugate named poly-(argilylaspartamide-co-aspartic) acid-ampicillin, gentamicin (PAA-AG). PAA-AG conjugate displayed excellent biocompatibility on human cell lines compared with free drugs, potentially enlarging their therapeutic window and safety, and suitable mucoadhesive characteristics which may help local treatments of mucosal infections. Studies on planktonic cultures of clinical and reference strains of S. aureus, P. aeruginosa, and E. coli revealed that PAA-AG holds a broad-spectrum antibacterial efficacy, revealing high potency in inhibiting the growth of the tested strains. More interestingly, PAA-AG exhibited excellent antibiofilm activity on both Gram+ and Gram- communities, showing inhibition of their formation at

subMIC concentrations as well as inducing the regression of mature biofilms. In addition, the physicochemical properties of this tool could ensure ease of use via a parenteral route for emergency management as well as by topical administration for the treatment of persistent infections of mucous membranes. Given the high biocompatibility and broad antibiofilm efficacy, combined with the opportunity for synchronous co-delivery, the PAA-AG conjugate could be a valuable tool to increase the success of ampicillin/gentamicin-based antibiotic multitherapy.

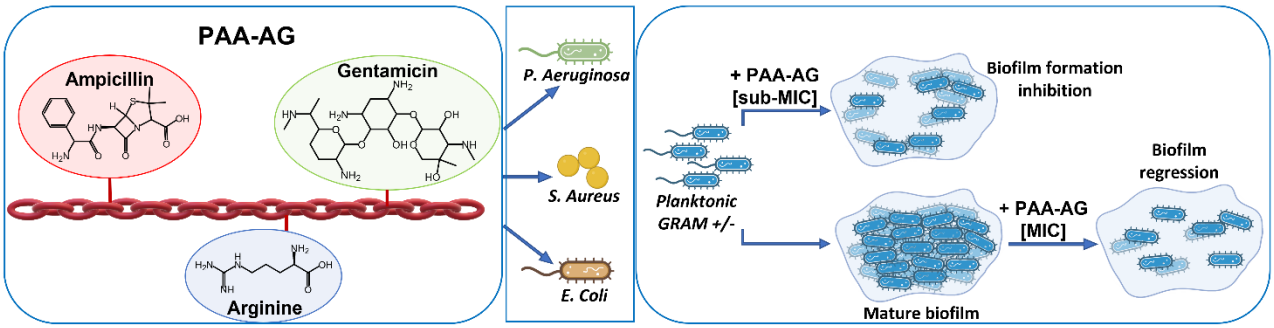

Figure 1. Schematic representation of the work

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Myelin nanovesicles as potential approach against neurodegenerative diseases

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ABSTRACT

Background: Neurodegenerative disorders (NDs) such as Multiple Sclerosis (MS), Parkinson and Alzheimer's disease are among the most serious health problems, degrading the quality of life and causing massive economic cost. The treatment of brain disorders and drugs administration routes remain, to date, a most interesting and attractive challenge for the researchers due to the presence of physiological barriers. In particular, the blood–brain barrier (BBB) and blood-cerebrospinal fluid barrier limits the accessibility to the brain and reduces the efficacy of therapies. Moreover, formulations selectively targeting specific brain regions and cells (neuron, microglia, or astrocytes), are essential to develop more effective therapies. Brain drug delivery systems can be a promising platform for overcoming these issues. Studies on nano-therapy for NDs have often focused on neurons. Nevertheless, the Central Nervous System (CNS) resident immune cells, such as microglia, are also emerging as a promising cellular target considering their principal role in neuroinflammation processes [1, 2]. In this scenario, we propose a new therapeutic strategy based on the bio-fabrication of **My**elin-based nano**Ves**icles (MyVes). MyVes, produced by microfluidic techniques, have a spheroidal morphology with a diameter of approximately 100 nm, negative zeta potential, and naturally contain myelin proteins [3]. Suitability of MyVes for passive or active drug loading procedures has been demonstrated. Furthermore, MyVes cross an *in vitro* BBB model, showing an enhanced tropism for the microglia cells in the white matter [3] (Figure 1).

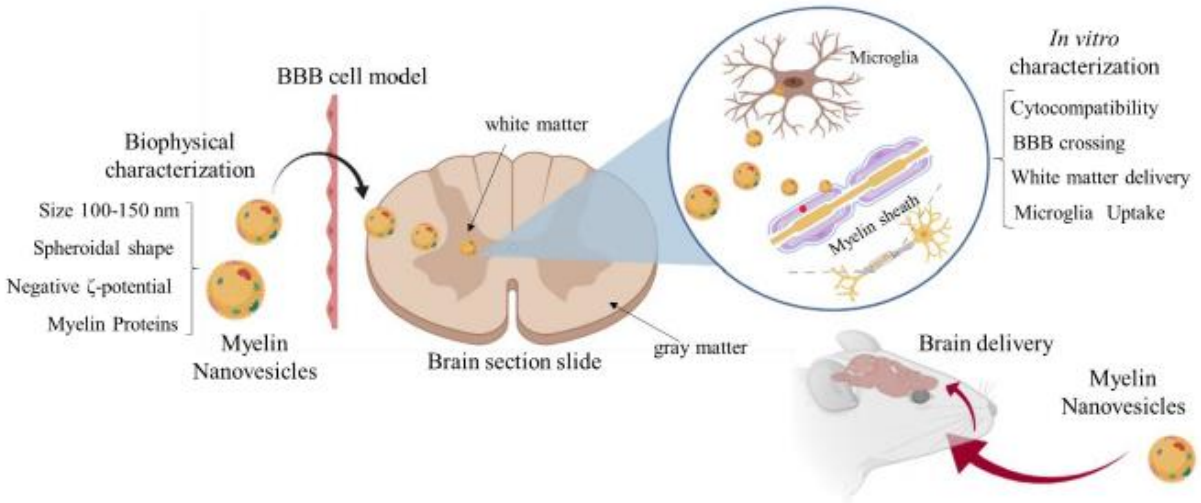

Figure 1. Biophysical and biological characteristics of the myelin nanovesicle

Results: we have tested and characterized the distribution and uptake of MyVes *in vivo* by intranasal administration. After injection, MyVes were able to reach the brain and localize in specific brain areas. By in *vitro test* we have demonstrated that the MyVes suppress the activation of microglia cells, reducing the neuroinflammatory process. Moreover, previous studies with myelin peptides (Antigens-Ags) have shown their safety, feasibility, and efficiency in inducing antigen-specific immune tolerance in MS [4]. Therefore, we have investigated the possibility that MyVes induce peripheral Ag-specific tolerance and suppress disease driven by an immune response against myelin Ags in peripheral blood mononuclear cells extracted to MS patients.

Conclusion: We propose a therapeutic strategy based on the bio-fabrication of new Myelin-based nanovesicles, through microfluidic procedure. Taken together, these data provide proof of concept for the applicability of MyVes as innovative brain drug delivery system to counteract white matter-related microglial diseases or for induce antigen-specific immune tolerance in MS.

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Sonochemical Synthesis and Characterization of Cetyltributylammonium bromide based Mesoporous Silica Particles

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ABSTRACT

Poor bioavailability of many drugs often impairs their therapeutic effectiveness, usually due to limited solubility and/or permeability. Numerous drug candidates fail to reach the market due to their poor pharmacokinetics. According to the Biopharmaceutics Classification System (BCS), orally administered drugs are divided into four classes based on their solubility/dissolution rate and permeability. Class IV drugs are characterized by low aqueous solubility, poor permeability, erratic and poor absorption, inter and intra-subject variability, and significant positive food effect, all of which results in low and variable bioavailability [1]. This problem has become a major challenge in drug formulation.

Mesoporous silica materials (MSMs) have been proposed as matrices for enhancing the apparent solubility and dissolution rate of various drug molecules due to their high surface area, regular pore structure, specific pore volume, and high thermal stability. Cetyltributylammonium bromide (CTBABr) is commonly used surfactant in various industries, including pharmaceuticals, cosmetics, and research laboratories. In this work, MSMs have been prepared according to the method reported earlier [2], with an additional step of sonochemical synthesis [3], and the CTBABr was used as a new potential templating agent. To adhere to the principles of green chemistry during the sonochemical process, the new matrices (SN1-10) were synthesized by combining non-ionic surfactant (Pluronic P123) with cationic surfactants (CTABr, CTBABr). The prepared matrices were characterized using particle size analysis, SEM,TEM and XRD analysis. The selected MSMs were then loaded with a model Class IV drug (furosemide) to assess their capacity to entrap and release an insoluble model drug. The resulting particles were analyzed for drug content, followed by a dissolution test.

The MSMs obtained during the synthesis process described in this work offer the possibility to improve the solubility and permeability of the BCS Class IV drugs. The proposed synthesis method offers several advantages, such as the use of safe solvents (methanol, ethanol), reduction of solvent volumes and shortened reaction time (from 48 to 2 h).

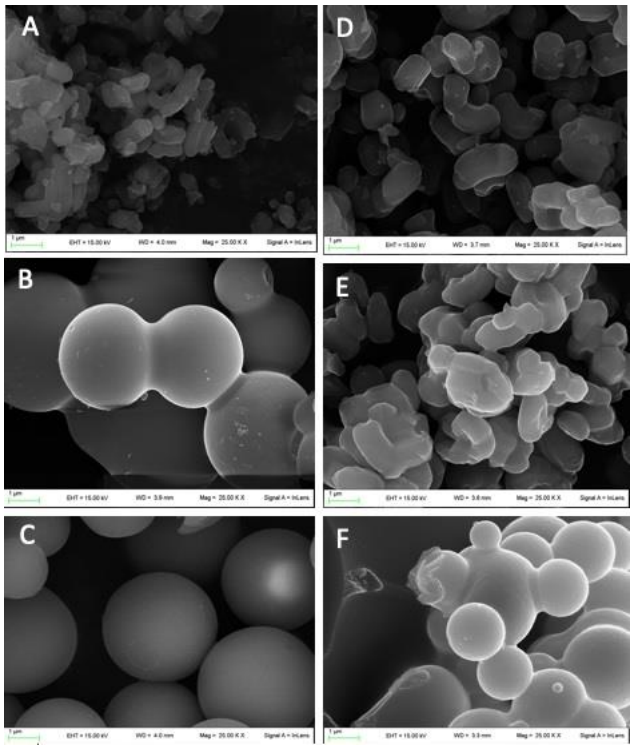

Figure 1: SEM (A-F) micrographs of matrices obtained by combining non-ionic surfactants P123 with CTABr A) SN1, B) SN2 C) SN3 and CTBABr D) SN6, E) SN8 and F) SN9.

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4D printed approach to manufacture multipurpose smart implants for breast cancer management

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ABSTRACT

Breast cancer is the second most common type of cancer, especially among women. Breast-conserving surgery is the principal treatment for early-stage breast cancer. However, the high rate of local recurrence and breast tissue loss following tumour resection, have a negative impact on patients and survivors [1- 2]. Furthermore, given the poor prognosis and the variability of individuals and malignancies, new patient-centred strategies are required. Taking these factors into consideration, the aim of this project was to develop a smart implant that should be inserted promptly after the tumour resection to provide an anticancer effect, without overshadowing the aesthetic point of view. For this purpose, a versatile 4D printed implant was manufactured using carboxymethyl cellulose sodium salt and cellulose nanocrystals. To achieve the aims, the implant was loaded with doxorubicin hydrochloride, and it was programmed to change the size, under swelling, to better fit in the tissue void. After an initial optimization of the formulation and the printing parameters (e.g., infill percentages and patterns), the smart implants were successfully produced and characterized. As pictured in Figure 1, the morpho transformation could be programmed through the choice of the infill pattern, allowing for better customization according to the patient's need. The mechanical properties and release studies were explored. Furthermore, the anticancer activity was tested on MDA-MB 231 cells, showing a reduction in the viability of -58% after 72h of incubation. Additionally, the anticancer effect was investigated after 4 weeks storage, showing the long-term efficacy and stability of the device. Overall, the results suggested that the 4D printed implant represents an innovative strategy for breast cancer management. Being able to fill the void in the breast resulting from the surgery and provide an anticancer effect to prevent recurrences [3].

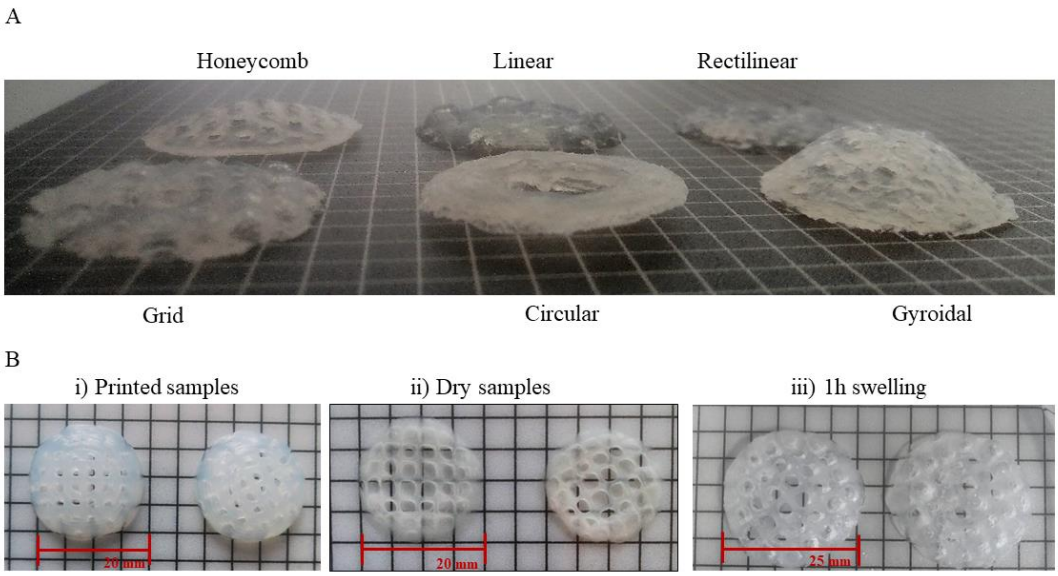

Figure 1: A) Lateral view of the swelled implants printed with different infill patterns. **B)** top view of i) printed designs ii) dry designs iii) swelled designs at 1 h timepoint of the implants manufactured with the grid pattern.[3]

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Unveiling the cisplatin/hyaluronan complex: a macromolecular delivery system for safer loco-regional chemotherapy

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ABSTRACT

Pleural Mesothelioma (PM) is a rare, incurable tumor with poor prognosis due to late diagnosis and frequent recurrences. Cisplatin (cisPt) is first-line systemic therapy, but the dose-limiting toxicity push clinicians to search for locoregional delivery. A cisPt-loaded hyaluronan (NaHA) film was studied for loco-regional chemotherapy of PM to improve the low effective procedure to wash the operated tissue with a cisPt solution. The biodegradable NaHA film, stuck on the operated thorax surface after surgical resection of PM, released cisPt to local tissues providing high drug concentrations for long time. Its anticancer efficacy was proven in a rat mesothelioma model (Fig. 1). In the pharmacokinetic study in healthy sheep, following film application, there were no signs of organ toxicity by cisPt vs. intravenous drug solution [2]. These unexpected results led to the discovery that cisPt was not circulating as such, but complexed to NaHA. SEC-HPLC analysis qualitatively evidenced the cisPt/NaHA complex in the film-forming mixture (FFM) because only NaHA peak was detected [3]. Atomic absorption spectroscopy confirmed cisPt co-elution with NaHA. The simultaneous quantification of cisPt and NaHA in FFM by RP-HPLC, showed that 5% of cisPt was free and 95% bound to the polymer. cisPt coordination to NaHA increased the viscosity of FFM, modifying its viscoelastic properties due to a cross-linking action. DSC proved the existence of cisPt/NaHA complex in the dry film: water evaporation T was lower than for a cisPt-free film.

cisPt release from the film was investigated in water and in 0.9% NaCl. There was no release in water and the film did not dissolve, substantiating Pt cross-links. In fact, release of cisPt from films occurred when Cl^- ions from the medium displaced Pt^{2+} from the complex with polymer. As the film also dissolved, drug and polymer existed as nanoparticles of cisPt/NaHA macromolecular complex that prolonged drug release up to 48h.

Finally, the anti-proliferative activity of cisPt/NaHA complex in comparison with cisPt alone and cisPt associated with the synergistic valproic acid (VA), was determined in lung epithelial (A459),

pancreatic cancer (MIA PaCa-2; $BxPC_3$) and melanoma (A375) cells by MTT assay after 48h incubation. The solution of cisPt/NaHA complex *in vitro* was as cytotoxic as cisPt alone, irrespectively of the presence of VA. The lowest IC_{50} was achieved in A375 melanoma cells, which form 3D spheroids. The complex, with and without VA, slowed down spheroid growth: the cisPt/NaHA film inhibited the development of spheroid-derived new cell colonies, which foresees the possibility to stop local tumour relapses. In conclusion, these results open to the possibility of delivery of cisPt/NaHA complex to tumors other than mesothelioma.

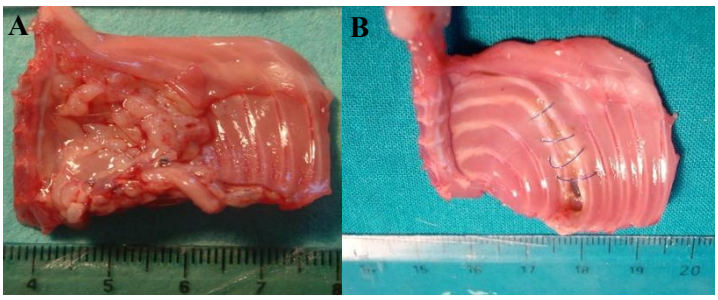

Figure 1. (A) Macroscopic appearance of a large tumor recurrence after 6 days after mesothelioma and lung resection and intrapleural application of cisplatin solution. (B) Macroscopic appearance of the chest wall 6 days after mesothelioma and lung resection and intrapleural application of cisPt-loaded hyaluronan film.

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Polyamidoamine decorated carbon nanodots as theranostic platforms for siRNA delivery

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ABSTRACT

The clinical application of siRNAs has attracted considerable interest in cancer treatment by offering the possibility to down-regulate target disease genes, maximizing therapeutic outcomes, and avoiding off-target and side effects typical of conventional cancer therapies. However, their use in the clinic is severely limited by their short half-life, rapid degradation by nucleases, and activation of innate immune responses[1].

Therefore, the development of multifunctional nanosystems represents a promising strategy for siRNA delivery to overcome the limitations in the use of viral vectors, allowing the release of the gene material exclusively into the target tissue[2].

The present work focuses on the design of hybrid theranostic nanosystems based on carbon nanodots doped with gadolinium and decorated on the surface with amphoteric polyamidoamines to combine the extraordinary theranostic properties of CDs[3] and the promising complexing abilities of polyamidoamines[4].

The CDs employed, synthesized from urea, citric acid and gadolinium under solvothermal conditions, showed high density of carboxyl groups on the surface, which were exploited to be functionalized with cystamine by amide coupling, resulting in CDs-Cystamine (CDs-Cys). Surface passivation with cystamine not only provided a redox-sensitive spacer for polyamidoamine binding but also improved the fluorescence performance of the nanosystem, due to a red-shift of the fluorescence profile.

In parallel, linear co-polymeric polyamidoamines based on methylenbis-acrylamide (MBA) carrying arginine and agmatine in side chain in different percentage ratios were designed, resulting in a set of random amphoteric co-polymers, with a different charge balance, homogeneous in terms of chain length, with high yields and reduced polydispersity.

After a systematic study of the siRNA complexing ability of the copolymers, the better-performing copolymer was selected for subsequent conjugation with CDs-Cys, choosing suitable conditions that allowed mono-addition of an unsaturated end of PAA to the terminal amine group of cystamine on the surface of CDs-Cys, resulting in a PAA/Cys molar ratio of 2:1.

Through electrophoresis assay, CDs-Cys-PAA conjugate has been shown to possess complexing ability four times higher than sole PAAs.

In addition, from stability studies in the presence of albumin, the nanosystem resulted stable to polyanion exchange, corroborating the possibility to administrate this system by systemic route, avoiding premature release of siRNA into the bloodstream.

Finally, through in vitro studies performed on cancer cell lines (HeLa and MCF-7) and the healthy line 16HBE, the cytocompatibility of both the obtained conjugate and interpolyelectrolyte complexes were evaluated.

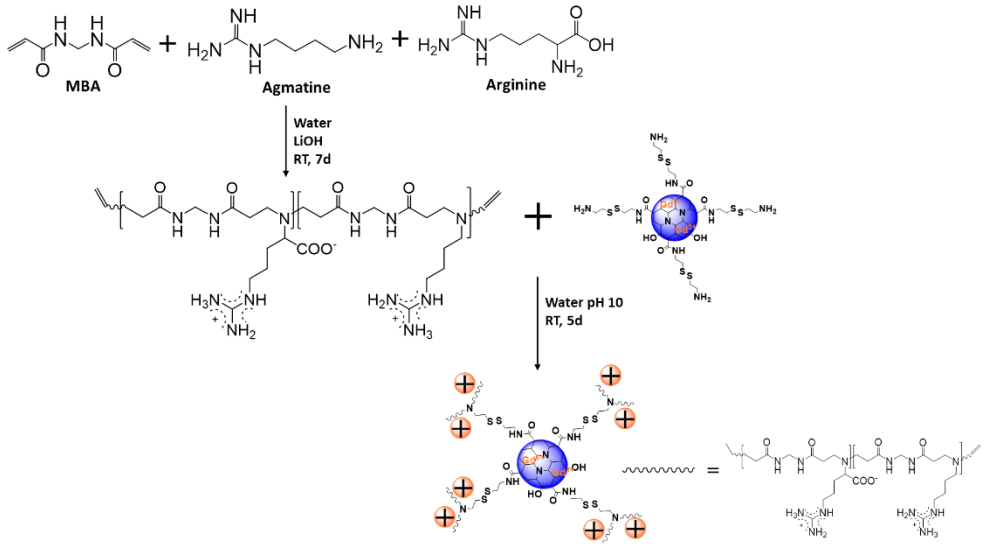

Figure 1. Synthesis pathway of CDs-Cys-PAA

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Acknowledgement

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Polybutilen succinate based electrospun fibrous scaffolds for ciprofloxacin sustained release.

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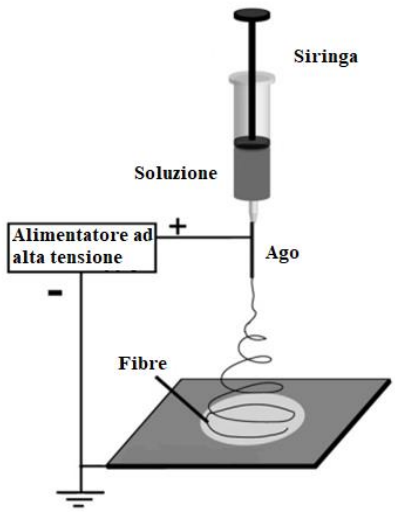
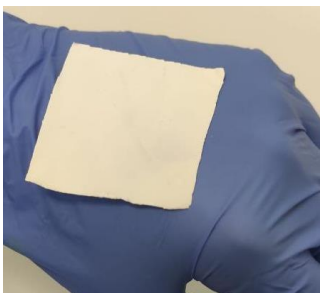
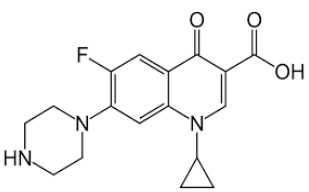
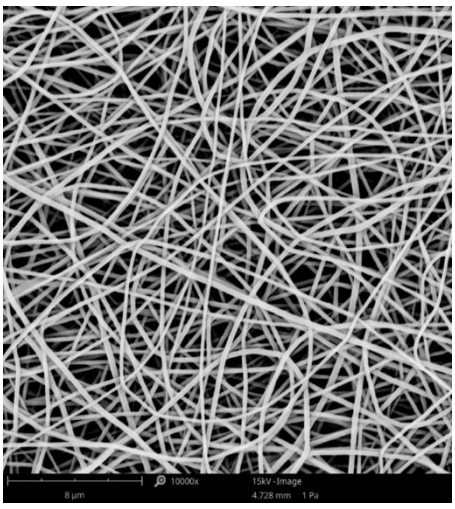
ABSTRACT

The release of antibiotic drugs from electrospun scaffolds is of increasing interest due to their ability to protect wounds against pathogens [Mtibe et al., 2023]. Electrospinning enables the production of porous systems that promote tissue regeneration and allows easy and flexible device manufacturing. In this study, we produced a material based on polybutylene succinate (PBS) loaded with ciprofloxacin (CPX) to achieve high local antibiotic concentrations, minimize systemic exposure, reduce administration frequency, and improve compliance [Tottoli et al., 2020]. Two scaffold of PBS (15% w/w), containing 5% and 20% of CPX (weight relative to the weight of PBS used) were prepared by electrospinning. SEM analysis, showed biomaterials consisting of fibrils with an average diameter of approximately 0.3 µm for both scaffolds. Experimental drug loading percentage (DL%) was calculated by solubilizing the scaffolds in chloroform, and the ciprofloxacin content was determined through UV analysis, which showed a DL% of 4.26% and 16.15%, respectively. ATR-FTIR analysis demonstrates that the active ingredient is incorporated within the PBS fibers.









Differential Scanning Calorimetry (DSC) analyses on the scaffolds, physical mixtures, the polymer and drug alone revealed that the thermograms of the physical mixtures (5% and 20% CPX) were

different from those of their respective scaffolds. Additionally, new peaks were observed in the scaffold thermograms, indicating the formation of chemical-physical bonds between the polymer and the drug during the electrospinning process. The permeation profile of ciprofloxacin (CPX) from the scaffold through pig skin was evaluated using Franz cells and compared to that of free CPX. The percentage of active ingredient permeated from the scaffold was higher compared to that of the free drug. After 24 hours, the scaffold exhibited permeation of over 40% of CPX, while the free CPX permeated less than 5%. The total amount of CPX contained within the scaffold permeated for over 80%, whereas the free CPX permeated only 16% of the total quantity after 72 hours. Finally, the antibacterial activity of the scaffolds was tested against the pathogenic bacteria S. aureus and P. aeruginosa. The results, expressed in colony-forming units showed significant differences in the antibiofilm activity of the CPX scaffolds compared to the scaffold without it. Results shown logarithmic reduction in bacterial growth. In conclusion we have prepared two PBS scaffolds able to release ciprofloxacin also trough pig skin and efficient as antibiofilm device, with potential application in wound healing.

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Nanomedicine combined with electrochemotherapy for a targeted cancer treatment.

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ABSTRACT

Electroporation (EP) is the application of a localized electric field able to increase the permealization of molecules into cell membranes. Due to the physically triggered phenomenon, the cell membrane can induce the temporary depolarization of the voltage-gated channels, which subsequent increases the cell permeability by hydrophilic pores formation (≈23 nm radius) [1]. The combination of EP with nanomedicines is starting to present itself as a valid adjuvant strategy for the treatment of some diseases, including head and neck, liver, pancreatic, and bone tumors[2,3].Therefore the designed molecular carriers (e.g., liposomes) of nanoscale dimension (hundreds of nm) should be able to guarantee an intracellular or extracellular (close to the target cells) drug-controlled release by application of external electric field. Moreover, thanks to the similarity between cellular and liposomal membranes, EP could be used as external trigger to obtain simultaneous and reversible electro-permeabilization that permits: i) easier uptake of the encapsulated drugs by the electroporated cells and ii) enhanced drug release into cytosol.

The aim of this preliminary work is to serve as a proof of concept to evaluate the response of the liposomal system when subjected to electroporation both from a morphological and functional (drug release) point of view. Liposomes were produced using microfluidic technique by NanoAssemblrTM platform (Precision NanoSystems Inc. Vancouver, Canada). DSPC:Cholesterol 50:50 and 70:30 molar ratio in ethanol solutions (10mM lipids concentration) were used for liposomes production. The flow rates ratio (FRR) and total flow rate (TFR) were set up as follow 8mL/min and 3:1 (aqueous phase: lipids phase)[4]. Starting from lipid compositions DSPC:Chol, DOTAP (cationic lipid) or DOPS (anionic lipid) were added until 5% molar ratio. Electroporator Gendrive IGEA (MU-GENEDRIVE_EN-Rev. 2.0 June 2021 equipped with UV-grade methacrylate cuvette (2mm) was used. Gentamicin sulfate (GS 1mg/mL in PBS, pH 7.4) was used as model drug for liposomes loading. Morphological characterization was performed through dynamic light scattering (DLS), Nano tracking analysis (NTA) and transmission electron microscopy (TEM). Encapsulation efficiency (EE%) and effect of electroporation on drug release were tested. Preliminary cell uptake on Human fibroblast (HNDF) was performed using fluorescent labeled liposomes.

The results showed that liposomes are in a dimensional range lower than 250nm with dispersion values between 0.2-0.3. EP, performed at increasing voltage values (160V, 200V and 250V) did not

alter the dimensions of the liposomes, thus not causing irreversible poration of the liposomes membranes (**Figure 1**). Charged liposomes, with 70:30 DSPC:Chol molar ratio, showed greater values of EE% (about 30 ± 5%) compared to 50:50 DSPC:Chol (about 10 ± 3%). Single cycle EP (Volt:160V, n° pulse:8, length:10μs, RT) performed on GS loading liposomes allowed to achieve a 46 ± 3 % GS release from liposomes. The cellular uptake of liposome undergoing EP, was faster compared to standard conditions (no voltage applied). Further trials are needed on tumor cell lines and/or bacterial cultures in order to exploit the nanocarrier-EP platform as a therapeutic strategy against resistant tumors or infections.

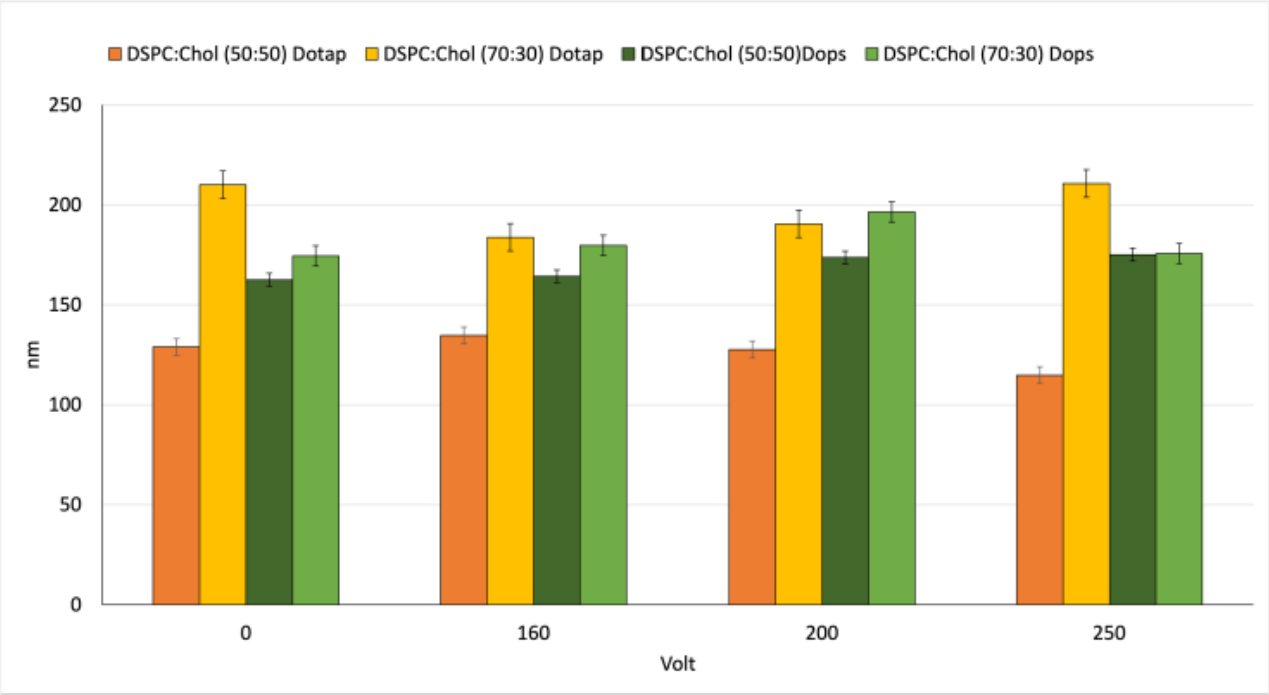

Figure 1: Effect of EP (160V, 200V and 250V) on liposomes morphology.

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Optimization of lipid nanoparticles loading CRISPR/Cas9 for gene editing

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ABSTRACT

Gene editing highlights as a promising strategy among nucleic acid-based therapies to target specific lung mutations. The Clustered regularly interspaced short palindromic repeats (CRISPR/Cas9) is a remarkable gene editing tool composed of a single guide RNA (sgRNA) and the CRISPR-associated Cas9 proteins. The system acts by precisely recognizing, cleaving, and repairing the targeted DNA[1]. Although the evidenced potential in targeting mutations, the direct delivery of CRISPR/Cas9 to lung cancer cells is challenging due to their strong instability in biological media. Lipid nanoparticles (LNPs) have evidenced their efficacy in delivering RNA to the cells and protecting the cargo from degradation[2]. The optimization of drug delivery systems is a key step to ensure high-performing formulations. This project aims to optimize LNP formulations to be further used as a platform to load CRISPR/Cas9 cargo and evaluate gene editing efficacy targeting KRAS, a frequent allele mutated in lung cancer.

mRNA-LNPs were prepared by microfluidics. A set of formulations with different lipids composition described in a ternary diagram were screened regarding particle size, PDI, zeta potential, encapsulation efficiency, and mRNA expression in a lung epithelial cell line. Subsequently, the best-performing LNPs were used to load sgRNA/mCas9. Both physicochemical characteristics and gene editing efficacy using the T7EI assay were investigated.

In summary, a total of 14 mRNA-LNPs composed of variable quantities of ionizable lipid, helper lipid, and cholesterol, but constant PEG-lipid concentration and N/P ratios were obtained. All formulations presented nanometric particle sizes, low polydispersity, and slightly neutral zeta potentials. The encapsulation efficiency was around 60% with non-significant statistical differences across all formulations. The eGFP expression for all LNPs outperformed the benchmark formulation and 3 LNPs demonstrated a significantly higher mRNA expression. Based on these outcomes, 3 LNPs were further manipulated with sgRNA/mCas9, ensuring similar physicochemical properties (**Fig. 1**). Finally, the gene editing efficacy targeting the KRAS lung mutation was successfully reported with comparable qualitative results as the positive control.

In conclusion, we demonstrated the optimization of LNPs for sgRNA/mCas9 delivery, ensuring particles with adequate physicochemical characteristics and an effective gene editing activity.

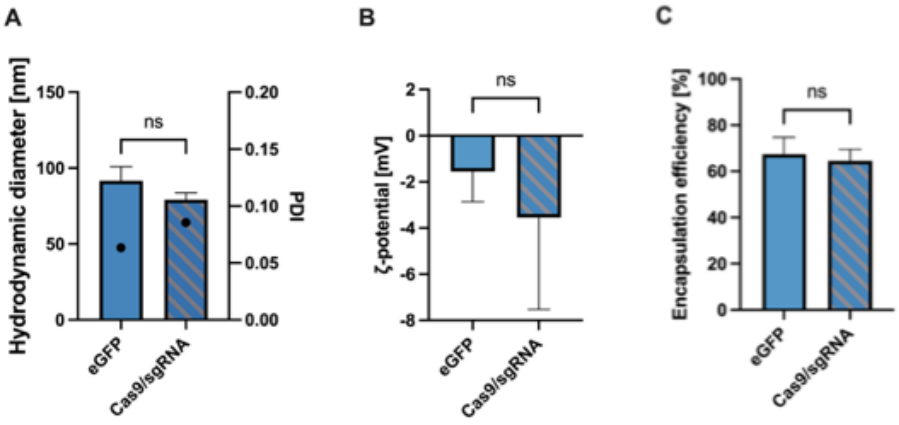

Fig. 1: Comparison of physicochemical properties between mRNA-LNP and CRISPR-Cas9-LNP regarding (A) hydrodynamic particle size and PDI, (B) zeta potential, and (C) encapsulation efficiency.

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Axonal transport of LNP for the treatment of neurodevelopmental disorders

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ABSTRACT

A variety of heterogeneous conditions are classified as neurodevelopmental disorders (NDD). These are characterized by an impairment of cognitive, behavioral and motor functions and often have overlapping phenotypes (Faundez *et al.*, 2019; Morris-Rosendahl and Crocq, 2020; Sang Cho *et al.*, 2019). Many NDD share an epigenetic dysregulation in their etiology, which ultimately affects the proper gene expression regulation during neural development. Lipid nanoparticles (LNP) are an effective tool for drug delivery, which has revealed optimal for gene expression modulation through the delivery of genetic tools such as mRNA sequences for protein expression and siRNA for targeted silencing (Kiaie *et al.*, 2022; Cui *et al.*, 2022; Cullis *et al.*2017). In this work, fluorescently labeled RNA loaded LNP were proven to be efficiently uptaken by primary rat cortical neurons through fluorescence microscopy and flow cytometry techniques. The retrograde axonal transport of LNPs was assessed by time-lapse microscopy of primary rat cortical neurons cultured in microfluidic chips allowing the fluidic isolation of the axonal and somal compartments and the local administration of LNP to the axonal termini. The present work will pave the way to efficient delivery of gene therapies for neurodevelopmental disorders, through the axonal uptake and transport of genetic tools-loaded lipid nanoparticles.

Nose to Brain delivery of NiR labelled PEG-PLGA/ PHEA-Dy700-PLA nanoparticles

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ABSTRACT

In the field of drug delivery, the nano-scale carriers offer unique advantages in terms of enhanced drug solubility, controlled release, improved bioavailability, and specific targeting. Their small size also allows the potential crossing of biological barriers. Thanks to these properties, nanostructured carriers have recently been proposed for the delivery of drugs to the Central Nervous System (CNS) through the Nose-to-Brain (N2B) route of administration, which offers a feasible passage from the olfactory or trigeminal pathways to the CNS, bypassing the blood-brain-barrier (BBB) (Musumeci et al., 2018). Polymeric nanoparticles (NPs) represent ideal release systems as they can be suitably designed and manufactured with features such as biocompatibility, stimuli-responsivity, dual or multiple drug loading, labelling with fluorescent dyes or specific ligands to increase their in vivo specific targeting/localization. In the present work, fluorescent polymeric NPs were prepared from two copolymers, the FDA approved polyethylene glycol-poly lactic acid-co-glycolic acid (PEG-PLGA), and the amphiphilic graft polyaspartamide/poly(lactide) copolymer, labelled by a near infrared (NIR) probe, the PHEA-g-Dy700-g-PLA (Craparo et al., 2021). The latter allows, thanks to the PHEA backbone, to stably conjugate molecules with various functions, such as markers and targeting agents. Furthermore, thanks to its amphiphilicity, by varying its concentration it is possible to modulate the characteristics of the particle core to optimise the drug loading of the incorporated drugs. The chemical-physical characterization showed that these NPs possess suitable mean size for administration through the N2B route (<200 nm), as well as good colloidal stability within 40 days after preparation, at different temperature, and stability in simulated biological fluids (such as simulated nasal fluid and cerebrospinal fluid). DSC analysis also revealed amorphous characteristics of prepared nanocarriers. By means of Fluorescence Molecular Tomography (FMT, Perkin Elmer) pre-clinical in vivo fluorescence imaging analysis were carried out in the near-infrared spectral

window to evaluate the localization of nanocarriers *in vivo* biological study on health mice after intranasal administration.

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Strategies to improve the delivery of chemotherapy in pancreatic cancer cells

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ABSTRACT

Pancreatic cancer (PC) presents one of the worst prognoses among all cancers with an aggressive and highly metastatic feature. Among the chemotherapeutics used to treat PC, gemcitabine (GEM) is the gold standard treatment, but, unfortunately, the molecule must be administrated at a very high dose because of its low halflife and poor biodistribution profile. Moreover, GEM resistance occurs within few months [1]. Many different approaches have been suggested to improve GEM uptake and metabolic stability, such as its encapsulation in liposomes or the synthesis of lipophilic prodrugs by linking linear acyl derivatives (*e.g.* stearoyl chain C18) [2].

In this study, GEM and C18-GEM were loaded into liposomes and the resulting nanoformulations were characterized. Then, their efficacy *in vitro* was evaluated and compared to free drugs. C18-GEM encapsulating liposomes (Lipo_C18) were prepared by the *thin lipid film and hydration* method and C18-GEM was directly included in the lipid film due to its lipophilic characteristics, whereas GEMencapsulating liposomes (Lipo_GEM) were prepared by ethanol injection method, exploiting drug's solubility in the aqueous *core*. Lipo_C18 and Lipo_GEM were characterized by size, zeta potential, and encapsulation efficiency (EE%). Both types of liposomes presented a diameter around 180 nm, a Zeta potential of -20 mV and an EE% of around 30%. The drug release profile of Lipo_C18 in HEPES buffer at 37°C showed that 30% of C18-GEM was released after 24h, demonstrating good stability of the formulation.

Previous studies demonstrated a higher cellular uptake of C18-GEM compared to GEM in the GEM-resistant pancreatic cancer cells Panc-1 and pancreatic cancer stem cells (CSCs). The increased cellular uptake resulted in an increase of cell death for both cell lines [3]. Therefore, our lipid nanosystem studies focused on C18-GEM and GEM-encapsulating liposomes. Lipo_C18 were tested for the *in vitro* cytotoxicity and compared to free molecules on both Panc-1 and CSCs. Lipo_C18 showed that the drug-loaded nanosystem was more toxic than the free drugs.

Overall, this study demonstrates that liposomes are useful nanosystems for improving the solubility, delivery and stability of GEM and C18-GEM. Our preliminary results are promising and will be further investigated. Lipo_GEM and Lipo_C18 toxicity will be further investigated in combination

with other drugs *in vitro*, with the purpose of improving their anticancer activity and overcome the resistance against GEM.

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Self-assembling nanoparticles for miRNA delivery towards precision medicine against melanoma

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ABSTRACT

Metastatic melanoma is a highly aggressive tumor with a poor prognosis [1]. The identification of a subset of patients whose mitogen-activated protein kinase (MAPK) pathway is overactivated has led to development of targeted therapy, which can inhibit this pathway. However, the long-term efficacy of targeted therapy is compromised by the onset of drug resistance. We have recently identified a panel of oncosuppressor microRNAs (miRNAs) able to prevent the development of drug resistance to targeted therapy [2,3]. Systemic miRNA delivery requires the use of nanocarriers to prevent degradation by endogenous nucleases and to facilitate intracellular delivery [4]. Furthermore, due to the rapid development of therapies based on precision medicine, there is an urgent need to develop a versatile nanoparticle platform enabling miRNA loading and delivery at the point of care. To address these challenges, we have developed self-assembling nanoparticles (SANP) with a calcium phosphate core enclosed by a lipid shell [5] as a novel miRNA delivery platform against drug-resistant melanoma. SANP are prepared before use and offer the possibility to encapsulate the miRNAs required for the specific patient, paving the way to personalized RNA-based therapies. We optimized the lipid shell composition and mixing ratios to achieve miRNA-loaded SANP, which showed hydrodynamic diameters below 200 nm, high miRNA encapsulation efficiencies, good colloidal stability in serum, and low haemolytic activity. *In vitro*, selected SANP formulations were able to effectively deliver miRNA, to inhibit the release of soluble tumor-promoting factors (i.e., transforming growth factor-β1 and vascular endothelial growth factor a), and to prevent the proliferation of two different cell lines of metastatic melanoma. When used in combination with targeted therapy, miRNA-SANP formulations could inhibit cancer cell proliferation in a dose-

response manner. These results demonstrate the potential of the SANP technology as a platform for miRNA delivery against metastatic melanoma with unprecedented design flexibility and great potential for rapid clinical translation which can be used in combination with targeted therapy to prevent the development of drug resistance.

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Lipid nanoparticles encapsulating miR182-3p for breast cancer treatment.

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ABSTRACT

Keywords: miR182-3p, lipid nanoparticles, target therapy, breast cancer, triple-negative breast cancer.

Triple-negative breast cancer (TNBC) represents about 15-20% of the total breast cancer and remains responding exclusively to the chemotherapy while more advanced therapeutic approaches are inefficient. In the research of novel therapeutic approaches for TNBC, abnormal levels of telomeric repeat-binding factor 2 (TRF2), a protein with a key role in the maintenance of telomere structure and function, have been correlated to the tumor development and progression. Interestingly, TRF2 levels can be modulated by a miRNA, named miR-182-3p, suggesting novel RNAbased anti-cancer therapies against TNBC [1]. In this perspective, lipid nanoparticles (LNPs) can be used for overcoming the poor biopharmaceutical profile of miRNA.

In this study we developed lipid nanoparticles (LNPs) encapsulating miR-182-3p to ensure its efficient delivery *in vivo.* LNP- miR-182-3p were prepared and fully characterized in terms of size polydispersity index (PI), superficial charge (ZP) and miRNA encapsulation efficiency. MiR-182-3p and LNP- miR-182-3p were tested *in vitro* in human cancer cells and finally delivered *in vivo* in TNBC mouse models. Results showed that LNPs- miR-182-3p had a hydrodynamic diameters < 200 nm and high miRNA encapsulation. Moreover, miR-182-3p was able to abrogate TRF2 expression, thus activating DNA damage and inducing apoptosis *in vitro*. Finally, the intravenous injection of LNPs- miR-182-3p impairs tumor growth in TNBC without important adverse effects being also able to cross the blood-brain barrier and reduce intracranial tumors, representing a powerful tool to treat metastatic brain lesions [1].

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Radionuclide therapy with accumulated nanobots reduces bladder tumor size in vivo

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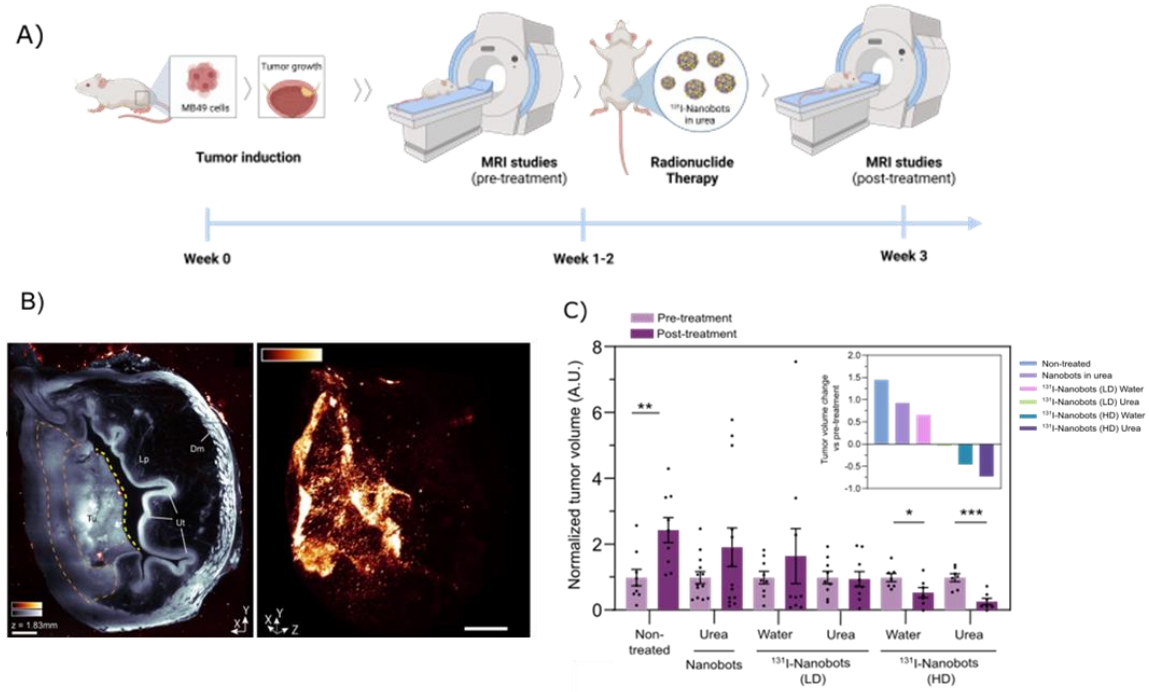
ABSTRACT

Enzyme-powered nanoparticles, known as nanobots, have emerged as a promising approach for performing tasks at the nanoscale, ranging from targeted drug delivery to precision medicine. Among these, urease-powered nanobots have shown improved diffusion and 3D navigation within biological environments[1] and drug delivery efficacy[2,3], compared to non-motile nanoparticles. The propulsion mechanism of these urease-powered nanobots, driven by urea (a readily available substance in the body), makes them particularly well-suited for potential applications in treating bladder cancer. Current treatments for this disease involve intravesical drug administration, which has shown good survival rates but limited therapeutic efficacy. Several factors, such as the sedimentation of therapeutic agents and the continuous addition of fresh urine, hinder the even diffusion of drugs

throughout the entire bladder volume. Moreover, poor retention in the bladder and low penetration in the target site may leave certain subregions untreated, potentially leading to recurrence. To address these unresolved medical challenges, nanobots have emerged as a viable solution. In this context, our study demonstrates an enhanced accumulation of radiolabeled urease-powered nanobots within bladder tumors using an orthotopic murine model. Furthermore, we provide evidence that intravesically administered radio-iodinated nanobots exhibit a radionuclide therapeutic effect, resulting in significant tumor size reductions of approximately 90% when compared with non-treated mice. These promising results firmly position nanobots as highly efficient nanosystems for bladder cancer therapy.



Nanobots penetrate and reduce bladder tumors size. A) Schematic representation of the radionuclide therapy studies. B) Left: Plane in the center of the bladder showing autofluorescence (grey) and scattered light-sheet (sLS) signal. Right: Maximum intensity projection of sLS signal inside the bladder. C) Normalized tumor volume obtained by MRI pre- and post-treatment. LD denotes low dose and HD high dose of ^{131}I.

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