18th School for Doctorate in Pharmaceutical Technology

INNOVATION IN LOCAL DRUG DELIVERY

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Associazione Docenti
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Scientific Program

SPEAKERS:

Bone regeneration: strategies when the frame fails

Franco Benazzo, IRCCS Policlinico S. Matteo, Pavia

Development of advanced drug delivery systems using natural based materials

Vitor M. Correlo, Universidade do Minho, Braga, Portogallo

Viscoelastic gels for intraarticular administration

Laura Mayol, Università degli Studi di Napoli Federico II

UV – Damage and Benefits

Steven Nisticò, Università degli Studi di Catanzaro

Lipid nanoparticles as skin drug delivery systems: main features and potential therapeutic application

Carmelo Puglia, Università degli Studi di Catania

Drug delivery to the nail: Challenges and recent developments

Begoña Delgado-Charro, University of Bath, UK

Treating pancreatic cancer with hyperthermia: Translational requirements

Adriele Prina-Mello, University Trinity College, Dublin, Ireland

Implantable anti-cancer drug delivery systems

David Stepensky, Ben-Gurion University of Negev, Beersheba, Israele

Nanomedicines for pediatric cancer therapy

Maria Blanco-Prieto, University of Navarra

The relevance of local drug delivery in lung pathologies

Federico Lavorini – Università degli Studi di Firenze

Lung delivery for fighting infectious diseases

Ruggero Bettini, Università degli Studi di Parma

Autologous limbal stem cell transplantation in patients with limbal stem cell deficiency

Giovanni Milazzo, Chiesi Farmaceutici Parma

Evolution of the legislation for biotechnological products in Europe

Aurora Botti, Chiesi Farmaceutici Parma

In vitro human 3D models and advanced culture systems

Investigating the metastatic cascade through vascularized 3D human models

Matteo Moretti, Istituto Ortopedico Galeazzi, Milano

Development of a delivery systems for a local administration – A case study

Torkel Gren, Recipharm, Solna, Sweden

Formulation and Stability of highly potent anticancer drugs

Irene Oddone / Giuliana Verdone, BSP Pharmaceuticals S.p.A.

PERSONAL INFORMATION

Family name, First name: *Mayol, Laura*

SCOPUS Author ID: *7006017258*

ORCID: *http://orcid.org/0000-0002-8869-6465*

Date of birth: *02/05/1976*

• EDUCATION

2004 **PhD** in Material, Chemical and Production Engineering, Biomaterials address (XVII cycle), at the University of Naples Federico II. The research project focused on the rheological properties of soft natural tissues and their biomimetic substitutes (Supervisor Prof. Luigi Ambrosio, coordinator Prof. Nino Grizzuti).

2001 **Degree** in Materials engineering (110/110) University of Naples, Federico II.

• CURRENT POSITION

2005 to date **Permanent Researcher** at the Department of Pharmacy, University of Napoli Federico II.

• SUPERVISION OF GRADUATE STUDENTS AND POSTDOCTORAL FELLOWS

2014 *PhD student (Simona Giarra) in "Scienza del farmaco" Department of Pharmacy, University of Napoli Federico II. XXX Ciclo.*

• TEACHING ACTIVITIES

From **2005/2006** to date ***Adjunct Professor*** in Drug loading and targeting (Degree in Pharmaceutical Biotechnology, University of Naples, Federico II).

From **2009/2010** to **2016/2017** ***Adjunct Professor*** in Pharmaceutical Technology and Legislation (Degree in Biotechnology for Health, University of Naples, Federico II).

From **2017/2018** to date ***Adjunct Professor*** in Materials Science and Technology I, II, and III (Degree in Cardiocirculatory Pathophysiology and Cardiovascular Perfusion, University of Naples, Federico II).

From **2017/2018** to date ***Adjunct Professor*** in Materials Science and Technology (Degree in Health Professions Sciences - Technical Assistance Area, University of di Naples, Federico II).

From **2017/2018** to date ***Adjunct Professor*** in Materials Science and Technology (Degree in Audioprosthetic Techniques, University of Naples, Federico II).

• ORGANISATION OF SCIENTIFIC MEETINGS

2015 *Thematic workshop of Controlled Release Society Italy Chapter: Micro and Nanotechnologies to Overcome Biological Barriers.*

2007 *International scientific conference "Innovation in Drug Delivery: From Biomaterials to Devices".*

• INSTITUTIONAL RESPONSIBILITIES

2014 *Member of Faculty Committee for PhD in "Scienza del Farmaco" Department of Pharmacy, University of Napoli Federico II.*

• PARTICIPATION IN SCIENTIFIC JOURNAL EDITORIAL BOARDS

2018 "The Open Biotechnology Journal" - BENTHAM *Open*-ISSN: 1874-0707.

• MEMBERSHIPS OF SCIENTIFIC SOCIETIES

2012 to date *Interdepartmental Research Centre on Biomaterials (CRIB), University of Naples Federico II.*

2011 to date *National Institute of Biostructures and Biosystems (INBB).*

2010 to date *Società Chimica Italiana (SCI).*

2005 to date *Association of Italian Professors and Researchers of Pharmaceutical Technology and Legislation (ADRITELF).*

• RESEARCH ACTIVITIES

The scientific activity of Dr. Mayol was essentially focused on two lines of research.

The first one deals with the design of biomaterials mimicking natural soft tissues and is based, in particular, on the study of the relationships between their macroscopic properties and molecular structure, through an analysis of rheological and transport properties. The possible applications in the biomedical field concern the replacement and/or repair of a natural tissue by implanting or directly injecting the biomaterial into the human body. Alternatively, the use the biomaterials can function as a three-dimensional support (scaffold) able to encourage the growth of new cells, and, therefore, new tissue identical to natural one (tissue engineering).

The second line of research aims to design and develop polymeric, lipidic or zeolitic materials for local and systemic delivery of drugs. Such systems must be properly designed in order to ensure adequate residence times of the drug(s) at the site of administration and a control over its(their) release kinetics. For drugs with limited water solubility, the use of cyclodextrins as water-solubilizing agents and modulators of release kinetics is also investigated. Drug delivery strategies are of special interest in the delivery of drugs presenting pharmacokinetics and/or bioavailability issues such as biotechnological drugs. Rational engineering strategies, along with polymer chemistry, physical and chemical characterization of the systems, allow overcoming some drawbacks that severely hamper their clinic attainment.

- **Research projects**

Principal investigator for a research project that attracted ***regional funding*** in 2007 and a member of funded groups within other National and European research projects (PRIN 2005, PRIN 2010, PON 2012 and FP5 GROWTH-GRD1-2001-40401).

VISCOELASTIC GELS FOR INTRAARTICULAR ADMINISTRATION

Laura Mayol
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Osteoarthritis (OA) is the most common type of arthritis and degenerative joint disease. Disease evolution is associated with cartilage damage and loss, bone outgrowth and attrition, subchondral bone alterations, synovial tissue inflammation and altered synovial fluid (SF) properties [1]. The SF is a biological fluid which fills the joint cavity and, due to its viscoelastic properties, is able to perform two essential functions such as the lubrication of joints and absorption of mechanical shock. It is essentially composed of water, proteins and hyaluronic acid (HA), which is its macromolecular component responsible for the viscoelastic properties of the SF. More in detail, SF of a healthy young man exhibits rheological behavior that is typical of an entangled network, namely viscous at low frequency (G'' > G') where it has to function as a lubricant of the joint and prevalently elastic at high frequencies (G' > G'') where it has to function as an elastic fluid, storing mechanical stress, and so protecting articular tissues from compressive damage. The pathological alterations that occur in joint diseases, such as osteoarthritis, lead to reductions in both HA molecular weight and concentration in SF and a resultant decline in SF viscoelastic properties [2]. This reduced SF viscoelasticity may accelerate and contribute further to joint degeneration and pain for the patient. In this context, viscosupplementation by intra-articular injection of HA based hydrogels is an established therapy for the treatment of knee OA, its goal being to restore the elastic and viscous properties of SF. An example of viscosupplementation product is an HA-hexadecylamide derivative that was studied for its ability to improve the viscoelasticity of SF through its successful integration and association within the SF. The percentage of amidation, the length of the amine pendant chain and the molecular weight of the starting HA molecules, of course influence the rheological properties of this amphiphilic derivative. An alternative to viscosupplementation is a local drug delivery by means of intra-articular injection. This method of drug administration minimizes the toxic effects of the drug(s) administered systemically, maximizing, at the same time, its(their) local effects. However, traditional oral drug(s), delivered via intra-articular injection, are limited by the lack of a sustained release. Injectable drug delivery systems, such as hydrogels, have been extensively studied for this kind of applications being minimally invasive and also providing an extended drug retention time and high loading efficiency.

References

[1] Maudens P, Jordan O, Allémann E. Recent advances in intra-articular drug delivery systems for osteoarthritis therapy. Drug Discovery Today, **2018**, in press.
[2] Borzacchiello A, Mayol L, Schiavinato A, Ambrosio L. Effect of a novel Hyaluronic acid derivative on equine synovial fluid viscoelasticity. J Biomed Mater Res A. 3, 92, 1162-70, **2010**.

BIBLIOGRAPHICAL SKETCH – PROF.CARMELO PUGLIA

Professor Carmelo Puglia was born in Catania in date 22/09/1974.
He has achieved the degree Pharmaceutical Chemistry and Technology in the academic year 1997/98 at the University of Catania (110/110).
From 1999 to 2002 he carried out is research activity as Research Fellowship at the Department of Pharmaceutical Sciences of the University of Catania.
From 2002 to 2015 he worked as Research Scientist in Pharmaceutical Technology at the University of Catania, Department of Drug Sciences.
Since March 2015 he is Associate Professor at the Department of Drug Sciences of the University of Catania.

Prof. Carmelo Puglia participated to the following financed research projects:

- PRIN 2010-11: Tecnologie avanzate per la veicolazione di molecole farmacologicamente attive attraverso le barriere biologiche dell'organismo.
- PON: "Di.Me.Sa." Valorizzazione di prodotti tipici della dieta mediterranea e loro impiego ai fini salutistici e nutraceutici.

Main academic employments are the following:

2009-2011 - member of the Permanent Commission for Teaching (CPDF) of the Faculty of Pharmacy;
Member of the Faculty Board of the Research Doctorate in "Neurosciences" (XXX cycle);
Member of the Faculty Board of the Research Doctorate in "Chemical Sciences" (XXXIII-up today);
2009-2012: Vice coordinator of the Degree Course in Scientific Information on Drugs (Department of Drug Sciences);
2018: Vice coordinator of the Degree Course in Pharmaceutical Chemistry and Technology (Department of Drug Sciences);
2018: Coordinator of the Management Commission for Quality Assurance of Departmental Research (AQC-RD) at the Department of Drug Science, University of Catania.

He is member of the following professional and academic association:

- ADRITELF (Associazione dei docenti e ricercatori italiani di Tecnica e legislazione Farmaceutica);
- Cofounder and member of Italian Nanopharmaceutical Network (INN);
- member of NANO-I - Research Centre on Ocular Nanotechnology - University of Catania, Catania;
- member of the Multidisciplinary Research Center for Diagnosis and Therapy of Fabry Disease and Organ Transplants

He held several teaching duties for the undergraduate courses of the Department of Drug Sciences:
In particular, he was teacher of Pharmaceutical Legislation, Chemistry of Cosmetic Products, Phytocosmetics and Classification and uses of medical devices and diagnostics (the last one for the Specialization School in Clinical Pharmacy). Currently he is teacher of "Pharmaceutical Technology and Legislation" for the undergraduate course in Pharmaceutical Chemistry and Technology and of "Legislation of Herbal and Health Products" for the undergraduate course in Applied Pharmaceutical Sciences. Both courses are part of the training offer of the Department of Drug Sciences.

Prof. Carmelo Puglia has authored and coauthored about 80 scientific publications on peer-reviewed international journals and about 30 conference papers (oral and posters). **The research themes carried out by Prof. Puglia covers the following topics**:

• Preparation, physical and chemical characterization of lipid nanoparticles (SLN and NLC) as innovative drug delivery systems.
• In vitro and in vivo assessment of the degree of percutaneous absorption of active ingredients in formulations intended for topical (cosmetic or dermatological products) or systemic (transdermal systems) applications.
• Study of innovative technological processes for the stabilization of active ingredients of natural origin and technological strategies aimed at increasing the bioavailability of botanicals.

He has co-founded the "Nanopharmanet", the Italian Network of scientists operating in the Pharmaceutical Nanomedicine and Nanotechnology fields. Since 2016 he is a member of the editorial board of the journal "Current Medicinal Chemistry".
In August 2017 he obtained the National Scientific Qualification as Full Professor (03/D2; SSD: CHIM09).
Prof. Carmelo Puglia h-index is 26; the citation number is 2153 (source SCOPUS).

LIPID NANOPARTICLES AS SKIN DRUG DELIVERY SYSTEMS : MAIN FEATURES AND POTENTIAL THERAPEUTIC APPLICATION

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The delivery of drugs and active agents to the skin by formulations containing nanoparticles is a topic of considerable current interest. A number of studies have shown important advantages of these nanostructure-based delivery systems over conventional formulations, although several unfavorable features such as cytotoxicity, scarce bioacceptability and complex industrial scaling up are documented too [1].

Lipid nanoparticles are innovative carrier systems showing the capability to put together the advantages of other nanometric carriers minimizing the problems associated with these vehicles. Lipid nanoparticles possess important features useful for topical delivery of drugs and active substances.

Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) are able to enhance drug penetration into the skin, allowing increased targeting to the epidermis and consequently increasing treatment efficiency and reducing the systemic absorption of drugs. The encapsulation of actives into the solid matrix increases the chemical stability of chemically labile actives. Furthermore, following skin application, lipid nanoparticles form an invisible film, which helps to repair and to reinforce the natural lipid barrier function of the skin [2].

The use of nanocarriers in topical drug administration has raised major concerns about their safety and toxicity cause to their dimensions, similar to those of biological active molecules, and therefore potentially able to cross biological membranes and interfere with biological processes.

Lipid based nanocarriers characterized by a complete biodegradation and a biocompatible chemical nature, seem to possess the best requisites to be defined “nanosafe carriers” [3].

This talk will be focused on the main features of lipid nanoparticles, in terms of therapeutic application and recent advancements, furthermore the importance of these systems in the topical delivery of drugs and active substances will be discussed as well.

References

[1]. Vega-Villa K, Takemoto JK, Yanez JA, et al. Clinical toxicities of nanocarrier systems. Adv Drug Deliv Rev, 60:929-938, 2008.

[2]. Pardeike J, Hommoss A, Muller RH. Lipid nanoparticles (SLN, NLC) in cosmetic and pharmaceutical dermal products. Int J Pharm, 366:170-184, 2009.

[3]. Puglia C, Bonina F. Lipid nanoparticles as novel delivery systems for cosmetics and dermal pharmaceuticals. Expert Opin Drug Deliv, 9(4): 429-441, 2012.

M. Begoña Delgado-Charro BIOGRAPHICAL SKETCH

Reader (Associate Professor) in Pharmaceutics. Department of Pharmacy and Pharmacology. University of Bath. UK

Dr Delgado-Charro graduated in Pharmacy (1986) and received her PhD in Pharmaceutical Technology from the University of Santiago de Compostela (1990, Spain). She was a MEC-Fulbright fellow & visiting Assistant Professor at the School of Pharmacy, University of California, San Francisco (1991-1993), where she specialized in the iontophoretic delivery of peptides across the skin. After having served as an academic at the Universities of Santiago de Compostela (1990-1997) and Geneva (1997-2004), Dr Delgado-Charro moved to Department of Pharmacy and Pharmacology, University of Bath in 2004 as a Lecturer and is currently a Reader in Pharmaceutics. She was a National Expert on Secondment at the European Medicines Agency (EMA) from October 2015 to September 2016. During this secondment in the EMA's Office of Science and Innovation Support, she was involved in "business intelligence in drug development" and "road-to-market" analysis.

Her research focuses on [1] transdermal and topical drug delivery by passive and iontophoretic means, [2] the development of optimized methods to treat nail diseases, such as onychomycosis and psoriasis, [3] non-invasive sampling for drug monitoring and pharmacokinetics, and [4] mathematical modelling to predict the accumulation of chemicals in the skin and to predict skin absorption. Her work has been funded by the Leo Foundation, the Parkinson's Disease Society UK, the Medical Research Council, the Swiss National Foundation, the NHS-NIC, the US-FDA and several pharmaceutical companies.

Dr Delgado-Charro is a Fellow of the Higher Education Academy. She is member of the International Association of Therapeutic Drug Monitoring and Clinical Toxicology (IATDMCT) & Committee on "Alternative Sampling Methods", the European Federation for Pharmaceutical Sciences (EUFEPS), the Academy of Pharmaceutical Scientists (APS) & Regulatory Focus group.

Dr Delgado-Charro has supervised 13 PhD students and currently supervises three PhD students. Her scholastic work (Scopus author ID: 7003434711; h-index:31) includes 97 peer-reviewed scientific articles, 10 book chapters, 137 congress abstracts, and several patent applications. She was an invited speaker at the Perspectives in Percutaneous Penetration Conference, the 11th World Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology, the Skin Forum, the American Association of Pharmaceutical Scientists (AAPS), IATDMCT meetings, and the Gordon Research Conference (GRC) on Barrier Function of Mammalian Skin; she will co-chair this latter conference in 2021.

Personal statement on the research activity: I believe that the pharmaceutical sciences and, in particular, improving our understanding of biopharmaceutics, drug delivery and formulation, plays a major role in the development of innovative, safe and efficient products for patient benefit. Furthermore, continued progress in these areas is essential for the sustainability of the pharmaceutical industry and for the provision of cost-efficient health interventions. Following my recent secondment at the European Medicines Agency, participation in collaborative projects with the US-FDA, and networks such as the Regulatory Focus Group of the Academy of Pharmaceutical Scientists, I have developed a good understanding of the manner in which advances in the pharmaceutical sciences are exploited by the Regulatory Agencies.

DRUG DELIVERY TO THE NAIL : CHALLENGES AND RECENT DEVELOPMENTS

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Psoriasis and onychomycosis have a relatively high prevalence and impact considerably the patients' quality of life. Systemic therapies are considered effective but may cause drug interactions and toxicity that could be avoided with topical therapies. Unfortunately, the latter have limited efficacy. It is believed that topical products fail to ensure therapeutic levels across the nail plate and the nail bed because of the low permeability of actives across this thick, keratinized structure. Thus, chemical and physical enhancement methods such as iontophoresis [1-2] and lasers [3] have been investigated for their potential to decrease the plate barrier. However, it is also recognized that poor formulation of topical nail products adds to the problem. The rapid metamorphosis of organic solvent-based lacquers upon application can lead to drug crystallization, hindering any drug partitioning into and diffusion through the nail plate. Thus, improving drug delivery to the nail also requires development of vehicles that ensure release and delivery of actives over long periods of time. A new strategy combining physical "poration" and improved formulation [4-5] aims to porate the nail plate to provide sequestration sites for formulations from which drug release can take place for longer periods of time. Another area of recent progress has addressed how solvents, chemical enhancers and water alter the nail microstructure as well as the structural differences between animal models, healthy and diseased (onychomycotic and psoriatic) nail plate [6-8]. In conclusion, optimized topical nail treatments should be based on rational formulation and better understanding of the alterations observed in the diseased states.

References

[1] Delgado-Charro, MB. Iontophoretic drug delivery across the nail. Expert Opin Drug Del, 9, 91-103 **2012**
[2] Benzeval I, Bowen CR, Guy RH, Delgado-Charro MB. Effects of iontophoresis, hydration and permeation enhancers on human nail plate: infrared and impedance spectroscopy assessment. Pharm. Res. 30, 1652-1662, **2013**
[3] Vanstone S, Cordery AF, Stone JM, Gordeev SN, Guy RH. Precise laser poration to control drug delivery into and through human nail. J. Control Release 268, 72-77, **2017**
[4] Flores FC, Chiu W, Beck RCR, da Silva CB and Delgado-Charro MB. Tioconazole-loaded cationic polymeric nanocapsules as ungual drug delivery systems. Int. J. Pharm. 535, 237-244, **2018**
[5] Chiu WS, Belsey NA, Garrett NL, Moger J, Price GP, Delgado-Charro MB, Guy RH. Drug delivery into microneedle-porated nails from nanoparticle reservoirs. J Control. Release. 20, 98-106, **2015**
[6] Chiu WS, Belsey NA, Garrett NL, Moger J, Delgado-Charro MB, Guy RH. Molecular diffusion in the human nail measured by stimulated Raman scattering microscopy. PNAS. 112, 7725–7730, **2015**
[7] Nogueiras-Nieto L, Gómez-Amoza JL, Delgado-Charro MB, Otero-Espinar FJ. Hydration and n-acetyl-L-cysteine alter the microstructure of human nail and bovine hoof: Implications for drug delivery. J. Control Release, 156, 337-344, **2011**.

[8] Cutrín Gómez E, Anguiano Igea S, Delgado-Charro MB, Gómez Amoza JL, Otero Espinar FJ Microstructural alterations in the onychomycotic and psoriatic nail: relevance in drug delivery. Eur J. Pharm. Biopharm. 128, 48-56, **2018**

Prof. David Stepensky

PERSONAL INFORMATION

Work:	Department of Clinical Biochemistry and Pharmacology and School of Pharmacy, Faculty of Health Sciences, Ben-Gurion University of the Negev, PO Box 653, Beer-Sheva 84105, Israel
E-mail:	davidst@bgu.ac.il
Web-site:	http://fohs.bgu.ac.il/homes/stepensky
Tel. & Fax:	+972-8-6477381 (w), +972-54-5349669 (cell), +972-8-6479303 (fax)
Birth date:	August 17, 1972 Birth place: Moscow, USSR

EDUCATION

1997-2002	PhD studies at Dept. of Pharmaceutics, School of Pharmacy, The Hebrew University of Jerusalem. Advisors: Prof. Amnon Hoffman, Prof. Michael Friedman. Thesis: Optimization of mode of drug administration according to pharmacodynamic rationale. Graduated Summa cum Laude.
1996-1997	M.Sc.Pharm. studies at Dept. of Pharmaceutics, School of Pharmacy, The Hebrew University of Jerusalem. Advisor: Prof. Amnon Hoffman. Thesis: Bisphosphonate pharmacokinetics and screening of bisphosphonate activity in a bone cancer model.
1992-1996	B.Sc.Pharm. studies at School of Pharmacy, The Hebrew University of Jerusalem.
1988-1991	Medicine studies at the medical faculty, The 2nd Moscow Medical College.

WORK EXPERIENCE

2016-	Associate Professor, Dept. of Clinical Biochemistry and Pharmacology, Faculty of Health Sciences, Ben-Gurion University of the Negev, Israel.
2015-	Member of the Committee for approval of Generic Drugs, Drug Registration Department, Pharmaceutical Administration, The Israeli Ministry of Health
2013-2016	Senior Lecturer (Assistant Professor), Dept. of Clinical Biochemistry and Pharmacology, Faculty of Health Sciences, Ben-Gurion University of the Negev, Israel.
2008-	Member of Licensing Exam Committee for Pharmacists and Assistant Pharmacists, Health Professions Licensing Department, The Israeli Ministry of Health
2007-2013	Lecturer (Assistant Professor), Dept. of Pharmacology, Faculty of Health Sciences, Ben-Gurion University of the Negev, Israel.
2005-2007	HHMI postdoctoral associate, Dept. of Immunobiology, Yale University, New Haven, CT, USA. Advisor: Prof. Peter Cresswell. Topic: Analysis of MHC class I peptide loading complex at the single cell level using novel fluorescence imaging techniques.
2003-2005	Postdoctoral associate, Dept. of Immunology, Weizmann Institute of Sciences, Rehovot, Israel. Advisor: Prof. Lea Eisenbach. Topic: Cytotoxic

	responses to glycosylated MUC1 and BA46-derived peptides for carcinoma treatment.
2003-	Scientific consultant, Pharmacokinetics & PK-PD modeling, Alcobra Ltd., BioBlast Ltd., CTS Ltd., LipoCure Ltd., Modigenetech Ltd., SoluBest Ltd.., Tzamal BioPharma Ltd., Vecta Ltd., etc.
2002-2003	Research associate, Dept. of Pharmaceutics, School of Pharmacy, The Hebrew University of Jerusalem.
2001-2003	Assistant Director, The Drug and Medical Equipment Research and Quality Control Laboratory, Israeli Defense Forces.
1996-1998	Researcher, Peptor Company, Rehovot, Israel.

RESEARCH SKILLS

- Preclinical and clinical pharmacokinetics and pharmacodynamics, PK/PD modeling (WinNonlin, ADAPT, MATLAB, Monolix)
- Drug delivery and targeting, controlled release dosage forms
- Drug absorption and permeability
- Fluorescence imaging (confocal microscopy, FRET, FRAP)
- Molecular biology, tissue culture, biochemistry, experimental immunology
- Analytical methods development and validation (HPLC, GC, etc.), quality control of pharmaceuticals and medical devices

RESEARCH INTERESTS

My research interests are focused on drug delivery and drug targeting for the purpose of optimization of drug treatment. The aim is to identify the major mechanisms and the rate-limiting steps of targeted drug delivery on multiple levels (within the single-cell, specific organ or tissue, and on the whole body level), and to design new drug delivery systems (DDSs) suitable for targeted drug delivery. Such DDSs would increase efficiency and reduce toxicity of drug treatment and are utterly required for several groups of therapeutic agents, including biopharmaceuticals (due to their limited permeability and stability) and anti-cancer drugs (due to their high cytotoxicity).

These research interests originate from my academic background in the fields of medicine and pharmaceutics, and specialization in pharmacokinetics, pharmacodynamics, and drug delivery/targeting during the PhD studies. Postdoctoral research experience expanded my insights in the fields of immunology, intracellular trafficking, and fluorescence imaging. Therefore, in my current research I'm applying multi-disciplinary approach to address specific aspects of drug delivery and targeting on the *in vitro*, *in vivo* and *in silico* levels.

Selected specific research topics are:

1. Intracellular (subcellular) targeting of drugs.

Many pharmacological agents act intracellularly and need to be endocytosed, and reach the site of action in specific organelle to exert their action. The cells interior is highly compartmentalized, and complexity of the cellular endocytosis and trafficking pathways leads to suboptimal magnitude and duration of pharmacological effects at the organelle of interest as well as to non-specific effects due

to exposure of additional organelles to the drug. Thus, attaining efficient and selective pharmacological effects for intracellularly-acting drugs requires development of specialized DDSs that should be targeted to specific organelle and deliver the drug in a controlled fashion. For this purpose, particle or vesicle (liposome)-based DDSs can be used, and intracellular targeting can be achieved by decorating the drug or the DDSs with organelle-specific targeting moieties. This approach relies on recognition of these moieties by the endogenous intracellular trafficking mechanisms and preferential delivery of the drug or the DDS into specific organelle.

2. Intra-tumoral disposition of anti-cancer drugs.

Solid tumors are characterized by complex and dynamic morphology and abnormal vascular network that limits the uptake of anti-cancer agents and their penetration to the deep layers of the tumor. Leaky tumor vasculature and dysfunctional lymphatic drainage can promote the enhanced permeability and retention (EPR) effect, extravasation and retention of molecules in the tumor tissue. Over the last two decades many drug delivery systems (DDSs) have been designed to overcome the pharmacokinetic limitation of anti-cancer agents, and improve the balance between their efficacy and toxicity. However, the tumor targeting efficiency of currently available DDSs remains low, formulative changes and new strategies for increased drug permeability and distribution are required. We investigate the barriers for delivery of anti-cancer agents to solid tumors, and the ways to increase cancer cell exposure to these agents, using advanced delivery systems (liposomes, local anti-cancer implants) that incorporate specific active components and promoter drugs.

3. Drug targeting to specific organs and analysis of drug disposition on the whole body level.

This research project is focused on analysis of the whole body disposition of small molecular weight drugs vs. biopharmaceuticals and DDS-encapsulated agents using pharmacokinetic modeling and simulation approach. The major objective is to identify the drug disposition processes and the critical formulation properties that limit the treatment efficiency of biopharmaceuticals and targeted DDS. Specifically, I analyzed the efficiency of brain-targeted drug delivery using different formulations, following systemic and intra-nasal administration, used bolavesicular DDS that can be enzymatically decapsulated in a triggered fashion for brain-targeted delivery of drugs, analyzed disposition of anti-TNF-α antibodies in rheumatoid arthritis patients. I assess the suitability of these findings for other biopharmaceuticals and their targeting to other organs and tissues, and consult several Israeli Biotech companies that develop new drugs and DDSs.

SELECTED PUBLICATIONS

1. Kozlovskaya L, **Stepensky D**. Quantitative analysis of the brain-targeted delivery of drugs and model compounds using nano-delivery systems. J Control Release 2013, 171(1):17-23.
2. Kozlovskaya L, Abou-Kaoud M, **Stepensky D**. Quantitative analysis of drug delivery to the brain via nasal route. J Control Release. 2014, 189:133-140.
3. Kaplun V, **Stepensky D**. Efficient decoration of nanoparticles intended for intracellular drug targeting with targeting residues, as revealed by a new indirect analytical approach. Mol Pharmaceutics 2014, 11(8):2906-14.

4. Kozlovskaya L, **Stepensky D**. Mechanisms of cell death induced by infusion sets leachables in in vitro experimental settings. Int J Pharm. 2015, 478(2):693-701.
5. **Stepensky D**, Rimon G. Competition between low-dose aspirin and other NSAIDs for COX-1 binding and its clinical consequences for the drugs' antiplatelet effects. Expert Opin Drug Metab Toxicol. 2015, 11(1):41-52.
6. Popilski H, **Stepensky D**. Mathematical modeling analysis of intratumoral disposition of anticancer agents and drug delivery systems. Expert Opin Drug Metab Toxicol. 2015, 11(5):767-84.
7. Kozlovskaya L, Popilski H, Gorenbein P, **Stepensky D**. In vitro toxicity of infusion sets depends on their composition, storage time and storage conditions. Int J Pharm. 2015, 489(1-2):285-93.
8. Maity AR, **Stepensky D**. Delivery of drugs to intracellular organelles using drug delivery systems: analysis of research trends and targeting efficiencies. Int J Pharm. 2015, 496(2):268-74.
9. Ruzov M, Rimon G, Pikovsky O, **Stepensky D**. Celecoxib interferes to a limited extent with aspirin-mediated inhibition of platelets aggregation. Br J Clin Pharmacol. 2016, 81(2):316-26.
10. Maity AR, **Stepensky D**. Limited efficiency of drug delivery to specific intracellular organelles using subcellularly "targeted" drug delivery systems. Molec Pharmaceutics, 2016, 13(1):1-7.
11. Maity AR, **Stepensky D**. Efficient subcellular targeting to the cell nucleus of quantum dots densely decorated with nuclear localization sequence peptide. ACS Applied Materials & Interfaces, 2016, 8(3):2001-9.
12. Popilski H, Abtew E, Schwendeman S, Domb AJ, **Stepensky D**. Efficacy of paclitaxel/dexamethasone intra-tumoral delivery in treating orthotopic mouse breast cancer. J Control Release, 2018, 279:1-7.

IMPLANTABLE ANTI-CANCER DRUG DELIVERY SYSTEMS

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Solid tumors are characterized by complex and dynamic morphology that limits the uptake of anti-cancer drug and their penetration to the deep layers of the tumor. New drug delivery systems (DDSs) are being developed to target anti-cancer drugs to the tumor and to enhance their therapeutic effectiveness. However, the tumor targeting efficiency of currently available systemically administered DDSs remains low, and only a small fraction of the systemically-administered dose (less than 0.7%) accumulates in the tumor due to the ERR effect.

Intra-tumoral (I.T.) administration can be used to deliver anti-cancer drugs and DDSs directly to the solid tumor, and limit their toxicity to other tissues. The majority of the organs and tissues are accessible for such administration, including such 'difficult' locations as the brain. Moreover, tight control of the drug release is possible by changing the composition and physical properties of I.T. formulations [1]. Despite these advantages, I.T. administration of anti-cancer agents exhibits low clinical effectiveness. Detailed analyses of the drug biofate following I.T. administration reveals that this finding is due to the limited intra-tumoral drug distribution, as only a thin layer of cells in immediate vicinity to the implant is exposed to the therapeutic concentrations of the anti-cancer drug [2]. Therefore, there is a need to improve the intra-tumoral pharmacokinetics of implantable anti-cancer formulations - to enhance the therapeutic permeability of the administered drug and effectiveness of the treatment [3].

In my lecture, I will describe the different types of implantable anti-cancer DDSs for I.T. administration, the barriers for anti-cancer drugs permeability and distribution from these implants into the solid tumors, and the approaches to increase exposure of cancer cells to these drugs using specific active components, promoter drugs, and external stimuli.

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BIOGRAPHICAL SKETCH

Maria J. Blanco-Prieto received her Pharmacy Degree from the University of Santiago de Compostela (Spain), followed by a PhD in Pharmaceutical Sciences from the University of Paris-Sud (France). She completed a post-doctoral training at the Swiss Federal Institute of Technology (ETH), Zürich, (Switzerland) and then joined the University of Navarra where presently she is Full Professor of Pharmacy and Pharmaceutical Technology.

She is the author or co-author of more than 125 research papers and book chapters, 4 editorials, 5 patents and over 160 communications at scientific conferences, many of them as invited speaker. She has been responsible of more than 25 competitive research projects projects and she is expert for the European Commission for nanomedicines. Her research in drug delivery has won her numerous awards. She is member of several international advisory boards and societies, editorial board member of several journals such as European Journal of Pharmaceutical Sciences, Cancer Letters, Molecular Pharmaceutics, etc.

Her research lay in the field of biomaterials and advanced drug carrier systems including the design and the development of polymer and lipid based micro- and nanoscale carriers, their biological evaluation in in vitro cell cultures (toxicity, mechanism of action, intracellular drug release) and also their pharmacokinetic and dynamic impact in vivo (using relevant animal models of the diseases). Research carried out in this field is mainly applied to cancer, cardiovascular and neurodegenerative diseases.

Nanomedicines in peadiatric cancers

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This talk is about the use of nanomedicines for pediatric cancer in the light of the dismal current situation in childhood cancer management. Compared to the situation with adults, agencies, governments and institutions have pointed to an alarming gap concerning the arrival of novel approaches in children. Indeed, chemotherapy protocols for childhood cancer are still problematic due to the high toxicity associated with chemotherapeutic agents and incorrect dosing extrapolated from adults. Childhood cancers, like adult cancers, have to receive special attention with a view to developing novel therapies that are fit for purpose.

Nanomedicines have emerged as novel candidates, especially for high-risk or relapsed patients when common drugs are unable to achieve success. The focus of this presentation will be to discuss the efficacy of orally administered edelfosine-loaded lipid nanoparticles for the treatment of pediatric osteosarcoma, the most frequent primary malignant bone tumor in the pediatric population [1-2].

Acknowledgements

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BIOGRAPHICAL SKETCH

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  Founder and Director, since its foundation, of the Interdepartmental Centre for Innovation in Health Products, Biopharmanet-Tec, University of Parma, Italy.
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- Education/training. Ph.D. in Pharmaceutical Chemistry and Technology, University of Pavia, Ital. M.S. in Pharmacy (Hons.), University of Parma, Italy.
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  2003-2017 President of the Master Course in "Pharmaceutical Technology and Regulatory Affairs", University of Parma.
  -2015-2016 Coordinator of the Doctorate course in "Biopharmaceutics-Pharmacokinetics", University of Parma.
  - 2012-2014 Director of the Italian inter-university Consortium Tefarco Innova;
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  - 2002- 2011 Associate Professor, School of Pharmacy, University of Parma, Italy.
  - 1997- 2002 Assistant Professor, School of Pharmacy, University of Parma, Italy.
  - 1994-1997 Postdoctoral Fellow, Department of Pharmacy University of Parma, Parma Italy.
  - 1993-1994 invited Visiting Scholar, School of Chemical Engineering of the Purdue University, West Lafayette, IN, USA.
  - 1999 J. Heller Journal of Controlled Release outstanding paper Award, Controlled Release Society, USA;
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  - 2014 Listed in "Who is Who in Thermal Analysis and Calorimetry", Akadémial Kiado, Hungary;
  - 2016 AAPS Fellow, American Association of Pharmaceutical Scientists, USA.
  - 1995 The Nagai Foundation Tokyo (Japan) Scholarship;
- Personal statement on the research activity
  Solid dosage forms for controlled and site-specific drug delivery, and solid-state chemistry for improving biopharmaceutical properties of active pharmaceutical ingredients: Nasal and pulmonary administration of powders; Particle engineering by spray drying and supercritical fluid technologies; Nano-sized systems for lung and oral drug delivery; Solid-state chemistry of drugs; Natural polymers for regenerative medicine applications; Technological aspects of the pharmaceutical production.

LUNG DELIVERY FOR FIGHTING INFECTIOUS DISEASES

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The delivery of antimicrobial agents to the lung in form of respirable microparticles represents an established approach for the treatment of infections caused by lung-resident pathogens. At least two products are already on the market, while several others are under development. The rational of this approach lies in the increased concentration of the active ingredient in the site of action with significantly reduced systemic exposure. Among the relevant infectious diseases, mycobacteriosis play a prominent role. According to the WHO 2017 Global Tuberculosis (TB) report, 10.4 million people are estimated to have fallen ill from TB in 2016 worldwide. On the other hand, non-TB mycobacteria are becoming a source of major concern in many geographical regions, as cause of superinfections in patients with immunodepression. Since mycobacteria resides into alveolar macrophages (AM) their efficient targeting requires both the capability to reach the deep lung with respirable particles and the maximisation of the intracellular drug concentration. The drug administration by inhalation through inhalable particles in the nanosize rage represents a strategy to increase drug concentration in the lung and reduce the systemic toxicity. These particles can enhance macrophage uptake as they may be engulfed by AMs. In order to optimize the process, two main issues need to be addressed: the high payload of the powder to be inhaled and the need to further implement the macrophage uptake through the use of specific chemical signals related to the nature of the material used for microparticle construction. Highly inhalable microparticles (aggregates of nanoparticles) made of hyaluronic acid of selected molecular weight to carry first line antibiotics for a more specific alveolar macrophage uptake are a novel anti-mycobacterial therapeutic regimen that could impact the resistant strains and shorten the duration of treatment. The selection of suitable efflux pump inhibitors to be associated to the antibiotics in order to increase the potency of these drugs and minimize the onset of resistant strains due to antibiotic selective pressure. On the other hand, infections may be prevented by immunization. In this respect, pulmonary delivery provides an attractive and alternative way for vaccine administration offering a great advantage over injection-requiring formulations, namely the possibility to reach an elaborate mucosal network of antigen presenting cells (macrophages, dendritic cells and B cells), that could induce the production of local antibodies (secretory immunoglobulins A) determining a broader protection compared to the exclusive production of serum immunoglobulins G induced by parenteral administration. From a technological point of view, the main problem that has to be solved is the transformation of the bioactive compound to be delivered (both the antigen and the adjuvant) into inhalable dry-powder particles. A related problem is the co-delivery of both the antigen and the immune-adjuvant as part of a single microparticle formulation, a mode of delivery that is known to enhance immunogenicity and ultimately the efficacy of vaccination.

BIOGRAPHICAL SKETCH

- Aurora Botti
- ATMPs & Biotechnologicals Regulatory Affairs Manager, Chiesi Farmaceutici S.p.A.
- Master Degree in Medical Biotechnologies
- Work in pharmaceutical industry since 2014 on regulatory development of new pharmaceutical products (ATMPs and Biotechnologicals) and drugs for rare diseases
- Active role in the Marketing Authorization Application for Lamzede, an Enzyme Replacement Therapy, designated as an orphan medicinal product
- Responsible and accountable for preparation, management and submission of regulatory procedure such as Priority Medicine (PRIME) Scheme and request and renewal of Orphan Medicinal Product Designation

EVOLUTION OF THE LEGISLATION FOR BIOTECHNOLOGICAL PRODUCTS IN EUROPE

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New therapies with genes, tissues and cells have taken the emerging field for the treatment of many diseases. Advances on stem cell therapy research have led the international regulatory agencies to regulate the development of new medicines with stem cells and to consider cell therapy products should be subjected to the same regulatory principles than any other medicine. In Europe, the Regulation EC 1397/2007 [1] established the rules for the advanced therapy medicinal products. With the new regulation introduced in Europe, the approach in the development of advanced therapies, usually developed by small and medium enterprises and academia (hospitals, universities, etc.), become a long and complex process. Regulation needs to be applied from the early stages of development of a new medicine to ensure that it meets the requirements of quality, efficacy and safety for administration in humans. Academic institutions and small and medium enterprises are not so familiar with regulatory issues as conventional pharmaceutical industry.

Holoclar®, an advanced therapy with limbal stem cells for the treatment of limbal stem cell deficiency was originally developed in 1998 by an academic institution as a surgical procedure [2] and used in more than 200 patients until 2007. Following the establishment of the EU ATMP Regulation EC 1394/2007, Holoclar was classified as a medicinal product and required that manufacturing was as per current Good Manufacturing Practice requirements and efficacy/safety has to be demonstrated for granting a marketing authorization. Holoclar was approved for commercialization in the EU in 2015 for the treatment of limbal stem cell deficiency due to ocular burn, a rare disease that can result in blindness. Holoclar was the first medicine containing stem cells approved in the EU.

This presentation describes how the new regulation impacted with the clinical use of Holoclar and the regulatory hurdles jumped to enable the translation of academic research and clinical experience into a pharmaceutical product compliant with the European Union regulations of Advanced Therapy Medicinal Products (ATMP).

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BIOGRAPHICAL SKETCH

Matteo Moretti is Head of the Regenerative Medicine Technologies Laboratory at the EOC, Lugano, Switzerland and of the Cell and Tissue Engineering Laboratory at the IRCCS Galeazzi Orthopedic Institute, Milan, Italy. Prior to this he worked as postdoctoral fellow at Massachusetts Institute of Technology, Harvard-MIT Div. of Health Science and Technology, Langer Lab. Both of his degrees, B.Eng (Politecnico di Milano) and research M.Sc (Trinity College Dublin, with Prof. P.J. Prendergast) are in Bioengineering. He obtained a European Ph.D in 2005 from Politecnico di Milano, pursuing his research also in Prof. I. Martin's Tissue Engineering Lab at U. Basel. His main research interests lie within osteochondral tissues and advanced cell culture technologies. In particular, on engineered tissues, 3D tumor models, tissue vascularization and in the study of tissue integration and vascularization aimed at translational applications. In multi-scale bioreactor systems from design to fully working prototypes, aimed at developing microfluidic and tissue bioreactor technologies for high-throughput and up-scalable, automated platforms as a key to more viable advanced therapies and in vitro 3D model systems for biology research. He is the treasurer of the Tissue Engineering and Regenerative Medicine International Society (TERMIS-UE) and acts as expert grant reviewer for several funding institutions, including Europe EU-FP7 / Horizon 2020, UK BBSRC, EPSRC and Cancer Research, Israel-US BSF, Hong Kong ITC, German BMBF, ZonMw and Dutch NOW and others. He is a reviewer for over 25 journals, including: Nature Biomedical Engineering, Scientific Reports, Biomaterials, ACS Nano, Drug Discovery Today, Nanomedicine and his awarded scientific prizes include a N.A.S.A. Tech Brief Award for development of scientific innovations. Industrially, he has been coordinator of EU Projects for Fidia Advanced Biopolymers; deposited 2 patents, of which one licensed to a company, and has been co-founder of 2 biotech start-ups (SKE srl and CELLEC Biotek AG) focused on bioreactor technologies. He is author of more than 65 publications in international peer reviewed scientific journals.

IN VITRO HUMAN 3D MODELS AND ADVANCED CULTURE SYSTEMS

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The discovery of new drugs against devastating diseases like cancer is a very relevant goal for the research and pharma industry, however, current models for drug discovery present severe limitations, leading to high attrition rates of new compounds. In particular, standard in vitro models propose an oversimplified microarchitecture as compared to native tissues, limiting the possibility to investigate complex biological processes. On the other hand, in vivo models, although widely used, suffer from species-specific differences in biological mechanisms, thus potentially generating misleading results. Advanced 3D in vitro models, based on human cells, have been proposed as a promising tool to overcome these limitations [1]. Among the techniques that can be exploited to generate such innovative models, microfluidic [2] and microfabrication [3] have gained increasing interest. In particular, microfluidics can provide useful model systems to investigate complex phenomena under combinations of multiple controllable biochemical and biophysical microenvironments, coupled with high resolution real time imaging. However, microfluidic systems involve very low cell numbers and tissue volumes, limiting the application of –omics analyses. On the other hand, higher scale in vitro models with a reliable mimicking of the in vivo tissue microarchitecture and biochemical milieu can be produced using microfabrication techniques, although with a lower resolution.

In this context, our group started to develop microfluidic and mesoscale systems to be used for the study of pathological mechanisms involving musculoskeletal tissues such as bone metastasis or muscle fibrosis. Those models are based on human cells embedded in a hydrogel, in different combinations depending on the tissue to be replicated. Considering that nearly all the physiological tissues present a network of blood vessels, it is imperative to reproduce vascularized tissues [4]. We thus concentrated on the obtainment of vascularized bone and muscle tissue models and exploited them for the study of processes in which vasculature plays a key role such as tumor cell extravasation or crosstalk between endothelium and skeletal muscle.

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INVESTIGATING THE METASTATIC CASCADE THROUGH VASCULARIZED 3D HUMAN MODELS

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Metastases of a primary tumor develop in specific organs depending on the tumor itself, e.g. breast cancer metastasizes preferentially to bone but not to muscle, through a multi-step process culminating with cancer cells (CCs) extravasation through the endothelium. To study such a complex phenomenon and to find drugs potentially limiting its occurrence, present models are not adequate.

In this context, we generated microfluidic and meso-scale vascularized models based on our already developed systems [1,2]. The microfluidic model was exploited to study the role of platelets and neutrophils in bCCs extravasation and the effects of $\alpha 2b\beta 3$ integrin inhibition. It involved the co-culture of fibroblasts and HUVECs embedded in a fibrin gel. The meso-scale models were used to investigate organ-specific bCCs behavior and effects of a known anti-proliferative drug (rapamycin). We developed a bone-like model with HUVECs embedded in a fibrin gel with osteoblasts, osteoclasts and macrophages and a muscle-like model with myoblasts and muscle fibroblasts.

In the microfluidic chip, bCCs extravasated more in the presence of neutrophils and platelets (49.99±3.39% vs. 22.66±4.75%, $p<0.001$) whilst the addition of $\alpha 2b\beta 3$ inhibitor (eptifibatide) reduced extravasation to 14.99%, by increasing anti-tumor effects of neutrophils and strengthening HUVECs junctions through decreased VE cadherin nuclear translocation. In the vascularized mesoscale models we found that microvascular networks were more developed in bone than in muscle tissue, and even more in presence of macrophages. bCCs were seeded in the bone and muscle models and their growth was significantly decreased in the muscle as compared to the bone model (50% decrease, $p<0.05$). Addition of rapamycin to the system reduced bCCs proliferation in a dose-dependent manner.

In conclusion, with the microfluidic model we highlighted the key role of platelets and neutrophils in bCC extravasation and we showed that inhibition of $\alpha 2b\beta 3$ integrin decreased their extravasation, identifying biological mechanisms involved in the anti- metastatic effect of an already approved drug. On the other hand, with vascularized mesoscale models we recapitulated organ-specific bCCs proliferation and response to known anti-tumor drugs.

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BIOGRAPHICAL SKETCH

Biographic sketch – Torkel Gren

Torkel Gren is Senior Director, Science Technology Officer at Recipharm AB, a Contract Development and Manufacturing Organisation (CDMO), with about 5 000 employees and operations in Europe Asia and North America. Torkel holds an MSc in Pharmacy and a PhD in Pharmaceutics from Uppsala University as well as a BSc in Business Administration from Mälardalen University. He has worked in the pharmaceutical industry since 1988 in companies such as AstraZeneca and Pharmacia. He has held a number of scientist and manager positions in Europe and the US. He was lead formulator and co-inventor of Detrol OD/Detrusitol SR. Torkel is vice chairman of the Swedish Pharmaceutical Society.

Torkel has a strong interest in pharmaceutical product development. In particular he is interested in how to use dosage form design in order to develop drug products that are offering therapeutic advantages as well as being profitable. In his current position at Recipharm he is involved in many challenging product development projects from small and large pharmaceutical products.

DEVELOPMENT OF DELIVERY SYSTEMS FOR LOCAL ADMINISTRATION – SOME CASE STUDIES

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Systemic drug delivery, in particular by the oral and intravenous route, has for a long time dominated drug administration. This is probably due to that systemic drug delivery offers efficient transport of many active molecules to almost all organs in the body. For this reason much effort has been focused on developing suitable technologies for systemic drug delivery. On the other hand local administration has the obvious advantage of maximizing the amount of drug at the site of action while avoiding extensive exposure to other parts of the body. Consequently, it is not surprising that the interest is increasing for new pharmaceutical products intended for local administration. The possibility of developing new products from existing molecules is probably also contributing to this interest.

Unlike systemic administration where the design of the dosage form is relatively independent of the site of action, drug products for systemic administration must be carefully adapted to the target organ. This means that development of new medicines for local administration offers many interesting challenges for formulators and other product development scientists.

These challenges may include various adaptions of conventional methods as well developing novel technologies such as isostatic pressing in order to produce slow release medications for local treatment [1]. This presentation will give practical examples of challenges in development of drug products.

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WHAT KIND OF PHARMACEUTICAL TECHNOLOGY RESEARCH IS NEEDED? – A PRODUCT DEVELOPMENT PERSPECTIVE

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Pharmaceutical technology research is a field that over the years has contributed to a great number of new drug products with significant therapeutic advantages. By using scientific advances from many different disciplines new products with unique properties have been invented. Usually, the development cost for these inventions are significantly lower than the cost for the development of a new active ingredient. Clearly, pharmaceutical technology is extremely valuable from a health perspective as well as from a commercial standpoint. However, pharmaceutical technology by itself is not leading to new products. In order to use technological progress during the development process we need to make a number of considerations. We need to think about things such as control methods, manufacture of clinical trial materials, production costs, intellectual property, regulatory affairs etc.

Sometimes restricting our thinking too much to what is available today will limit progress but if we want pharmaceutical technology to generate new products in the short-term perspective it is important to understand the context of pharmaceutical product development. A new product must be possible to manufacture at a reasonable cost and it must be possible to register. This presentation will discuss pharmaceutical technology research from a product development perspective.

PhD STUDENTS PRESENTATIONS (ORAL PRESENTATION)

Electrospun nanofibers for tissue regeneration combined with drug delivery

Silvia Pisani – Università degli Studi di Pavia

A new wound dressing based on Spanish Broom fibers loaded with Vitamin E and Lactobacillus plantarum for skin wounds

Barbara Giordani - Università degli Studi di Bologna

Curcumin and resveratrol loaded electrospun nanofibers as wound dressings

Dalila Miele – Università degli Studi di Pavia

Brain delivery of Pt(IV)-prodrugs using pegylated solid lipid nanoparticles

Ilaria Arduino, Università degli Studi di Bari "Aldo Moro"

In search of smart lipid-based vesicles and smart triggers

Martina Nardoni, Sapienza Università di Rome

Supramolecular Hydrogels of Heterochiral Tripeptides

Evelina Parisi Università degli Studi di Trieste

Optimization and chemical-physical characterization of ASCn liposomes loaded with khellin

Laura Risaliti - Università degli Studi di Firenze

***In situ* gelling systems for the local delivery of natural compounds in the treatment of oral mucositis**

Barbara Vigani - Università degli Studi di Pavia

Escin-based nanovesicles to improve berberine topical delivery

Giulia Vanti - Università degli Studi di Firenze

Formulation development of a biopolymer based multilayer film for the local treatment and wound repair of periodontitis

Karthik Neduri - Università degli Studi di Genova

Pharmaceutical development of extracellular vesicle formulations: from mesenchymal stem/stromal cells to cell secretome in regenerative medicine

Elia Bari, Università degli Studi di Pisa

Development of innovative FDM-3D-printing technologies for the production of patient-tailored medicines

Marilena Saviano, Università degli Studi di Salerno

Oral spray congealed microparticles for the local intestinal delivery of biologics

Serena Bertoni, Università degli Studi di Bologna

Maltodextrins as drying auxiliary agent for the preparation of easily resuspendable nanoparticles

Giulia Magri, Università degli Studi di Milano

The effect of chitosan coating on albumin nanoparticles for nose-to-brain delivery

Vieri Piazzini, Università degli Studi di Firenze

Formulation and delivery of disease-modifying drugs as nasal powders for Alzheimer's disease

Laura Tiozzo Fasiolo, Università degli Studi di Parma

Development of Novel Nanomicellar formulations for the Ocular Delivery of Cyclosporine A

Eleonora Terreni, Università degli Studi di Pisa

ELECTROSPUN NANOFIBERS FOR TISSUE REGENERATION COMBINING SYNTHETIC AND NATURAL POLYMERS

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Electrospinning is an interesting technique able to produce polymer membranes made of entangled nanofibres. The technique is raising interest in the pharmaceutical and biomedical areas. Either electrospun membranes are studied for tissue regeneration purposes or for API controlled release [1], [2].

In this work suspensions of hydrochloride chitosan (CL113) in copolymer polylactide-co-polycaprolactone (PLA-PCL 70:30) solution were electrospun in order to assess polymer nanofibre blend membrane loaded with chitosan polymer. The membranes were crosslinked with Tripolyphosphate (TPP) in order to improve chitosan stability in aqueous environment. The aim of the work was to investigate the properties and stability of chitosan/PLA-PCL electrospun membranes considering their application for tissue regeneration. The electrospun membranes were characterized for their physico-chemical (FT-IR) morphology (SEM) and *in vitro* biological properties (cytocompatibility and cell engraftment).

Peaks and bands of the raw components have been detected in spectra of PLA-PCL/chitosan physical blending and are visible also in the FTIR spectra of PLA-PCL/chitosan membranes even if the signals of chitosan are less intense.

Uncrosslinked PLA-PCL/chitosan membranes show regular fibres with mean diameter of 700 nm; while crosslinked membranes film interconnecting fibres are evident. These data could probably due to TPP reacting with chitosan polymer distributed on fibre surface.

Results of cells viability on PLA-PCL/chitosan nanofibre membranes proved that cells gradually growth on PLA-PCL/chitosan nanofibre reaching almost 95 % at day 3. No evidence of cytotoxicity has been observed during the study. SEM analysis carried out after 3 days of incubation confirmed Ffbroblasts homogeneous growth and spread on PLA-PCL/chitosan nanofibre surface.

Previous researches demonstrated that PLA-PCL nanofibers membranes were suitable support for tissue regeneration.Blending chitosan with PLA-PCL copolymer led to improve PLA-PCL membrane hydrophilicity and cell attachment and proliferation, without losing the entangled nanofibres structure and stability.

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A new wound dressing based on Spanish Broom fibers for the local release of Vitamin E and *Lactobacillus plantarum*

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In the medical and pharmaceutical wound care market, cotton gauze is a traditional dressing and is still one of the most widely used product. Unfortunately, cotton cultivation requires an intensive use of pesticides and a large amount of water, leading to environmental problems. Taking into account these disadvantages, we investigated the potential use of Spanish Broom (SB) fibers that, as well as cotton fibers, are composed of cellulose, are widespread and have hydrophilic character. Furthermore, SB fibers can be extracted by an easy, efficient and fast physical-chemical process [1].

In this work, a new wound dressing based on SB fibers loaded with Vitamin E and *Lactobacillus plantarum* (LP) was developed as a new drug delivery system for the treatment of skin wounds. We used Vitamin E as natural antioxidant and LP was selected for its strong antimicrobial activity against *S. aureus* and *P. aeruginosa*. Vitamin E and LP were dispersed in sodium alginate (0.3%), a polymer that shows good properties, such as low cost, easy availability, biocompatibility and capacity to enhance healing of wounds. The obtained emulsion was then deposited on SB wound dressing and freeze-dried. The formulation was characterized in terms of drug loading, morphology, *in vitro* drug and lactobacilli release and viability during storage conditions. Cotton dressing was used as control. Vitamin E and LP loading was found to be 65.35 % and $8x10^{10}$ CFU/g, respectively. Scanning electron microscopy showed that freeze-dried emulsion can be easily spread in the SB and cotton fibers. Both SB and cotton wound dressing provided a sustained release of Vitamin E and LP, suggesting that they can be retained for a long time in skin wound assuring the healing process without the need of frequent replacement. Interestingly, compared to the cotton, SB wound dressing allowed higher release of both Vitamin E and LP, probably thanks to the ability of SB fibers to absorb great amounts of aqueous media. Moreover, LP showed a good stability over time for at least 8 months ($> 10^9$ CFU/g). In conclusion, the new wound dressing based on SB fibers can be used for local release of Vitamin E and LP. Moreover, SB can successfully replace the cotton in wound care.

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CURCUMIN AND RESVERATROL LOADED ELECTROSPUN NANOFIBERS AS DRUG DELIVERY SYSTEMS AND WOUND DRESSINGS.

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Bioactive dressings are designed to interact with the wound surface providing an optimal micro-environment and releasing bioactive molecules to accelerate the healing process. To get an effective action, some essential characteristics have to be considered, such as the type of wound, the wound healing rate and the physical - chemical properties of the materials employed.

Electrospun nanofibers can be employed as excellent wound dressing devices; in fact, through the combination between natural and synthetic materials it is possible to obtain systems characterized by a porous nature particularly suitable to drain the exudates released by the lesion, to allow an adequate gas exchange and to prevent microbial attack, which could lead to a delayed healing.

The aim of this work is focused on the design of electrospun nanofibers as substitutes of biological tissues that mimic the natural structure of tissues by interacting selectively with cells through specific biomolecular recognition.

The electrospun nanofibers are based on a mixture of native soluble collagen with a synthetic and biocompatible polymer (polycaprolactone - PCL). As bioactive molecules potentially relevant in the tissue regeneration process, two polyphenols were loaded separately into these systems: curcumin and resveratrol. Both have important anti-inflammatory and antioxidant properties and can be therefore useful to accelerate and to promote the repair of damaged tissue.

Collagen and PCL were mixed together in a 1: 1 weight ratio in acetic acid 90 % v/v as solvent. Either curcumin or resveratrol was added to the polymer solution at a final 0.5% w/w concentration. In the first phase of the study the collagen/PCL solutions unloaded and loaded with polyphenols were characterized by rheology, conductivity and surface tension. The solutions were electrospun and the nanofibrous membranes obtained were evaluated by morphology and nanofiber dimensions by means of SEM analysis, that indicated a regular structure and dimensions quite different according to the loaded polyphenol. Mechanical analysis was carried out using a texture analyzer on nanofibers dry and after hydration, demonstrating the stability of the structural integrity until 7 days in aqueous environment. Both for curcumin and resveratrol about 70% of the bioactive molecules was released from electrospun nanofibers during the first 6 hours, in line with the possible employment on wounds. Biocompatibility (by MTT test) and cell adhesion (by SEM and CLSM analysis) evaluation, performed on normal human fibroblasts of the dermis (NHDF), indicated for both the loaded systems good promotion of vitality and cell growth.

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BRAIN DELIVERY OF PT(IV) PRODRUGS USING PEGYLATED SOLID LIPID NANOPARTICLES

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Glioblastoma multiforme (GBM) is the most malignant form of gliomas causing death in patients few months after diagnosis. The treatments involve firstly, if possible, the surgical resection of the tumor followed by radiotherapy and finally systemic chemotherapy. Cisplatin is a potent chemotherapeutic agent, but its use for the treatment of brain tumors is limited by severe systemic toxicity and inefficient penetration of brain tumor tissues. To overcome this limitations Kiteplatin, the Pt(II) drug analogous of cisplatin, and its Pt(IV) prodrugs have been developed and evaluated for their activity against both cisplatin- and oxaliplatin-resistent tumors [1]. Furthermore, in order to maximize brain drug delivery solid lipid nanoparticles (SLNs) have been used as Pt(IV) prodrugs nanovectors. Great potential lies in the application of SLNs as drug delivery systems, thanks to different advantages, such as small and controllable size, stability, easy surface functionalization, no toxicity and biodegradation. One more advantages of the SLNs is that they reduce the toxicity of the therapeutic molecule that they transfer protecting them, at the same time, from reticuloendothelial system clearance. In this contest, quantum-sized-carbon-dots (C-dot) were used for the realization of optically traceable nanosystems able to visualize the biodistribution and storage of the carriers into the body. SLNs containing Pt(IV) prodrugs and C-dots have been prepared by a hot homogenization technique using cetyl palmitate as lipid matrix and polyethylene glycol modified phospholipids (PEG lipids), in order to achieve a PEG-based anti-fouling coating on SLN surface. These nanoformulations proved to be stable in aqueous medium and are characterized by a Z-average under 100 nm, a polydispersity index below 0.2, a zeta potential between −20 and −25 mV and a drug encapsulation efficiency in the range of 30-40%. *In vitro* cytotoxic effects and cellular uptake have been assessed in both human cerebral microvascular endothelial cells (hCMEC/D3) and in human glioblastoma (U87) cell line. Moreover, the *in vitro* brain permeability has been estimated on a human blood-brain barrier model using transwell devices with hCMEC/D3 monolayers.

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IN SEARCH OF SMART LIPID-BASED VESICLES AND SMART TRIGGERS

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A focus of current research is the development of innovative carriers able to respond to an external stimulus for on-demand therapeutic delivery. Within this context, in our laboratory, Fe_3O_4-in-liposome hybrid nanocostructs were prepared for remotely-triggered drug release. In particular, we demonstrated that magnetic nanoparticles (MNPs), integrated within liposomes, transmit mechanical stresses to the bilayer and, in this way, induce controlled release of the loaded drug when exposed to alternating (AMFs) or pulsed (PEMFs) electromagnetic fields, without temperature increase [1,2]. In a different approach, it was demonstrated the capability of activating lipid-based nanosystems by means of electromagnetic fields (EMFs). Specifically, it was analyzed the possibility of applying an electric trigger to remotely modulate the release from liposomes. The electrical stimulation could open the way to controlled release mediated by nanoelectropermeabilization, which could facilitate direct drug release to cells [3]. Finally, in order to further improve the performance of liposomes, polyethylene glycol-dimethacrylate (PEG-DMA) was photopolymerized within the inner core of hydrogenated soybean phosphatidylcholine and cholesterol (HSPC/Chol) vesicles with the intent to modify their liquid core into a soft hydrogel [4]. The polymer-phospholipid interactions existing in the new Gel-in-Liposome (GiL) system affect both the stability of liposomes and the membrane permeability properties.

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EFFECTS OF CHIRALITY ON TRIPEPTIDE SELF-ASSEMBLY

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Tripeptides are very promising simple building blocks to attain nanostructured hydrogels through self-assembly based on non-covalent interactions.[1,2] The insertion of a D-amino acid in a tripeptide sequence is sufficient to promote the achievement of stable peptide hydrogels, when the L-analogues don't gel.[3] Appropriate choice of amino acid chirality is thus a powerful tool to guide self-assembly and, in particular, the choice of the order of the amino acids in a hydrophobic tripeptide sequence allows fine-tuning of supramolecular order, which is reflected on hydrogel viscoelastic properties, and durability at physiological conditions.[3] Assembly of a tripeptide (^{D}-Leu-^{L}-Phe-^{L}-Phe) and drugs (Fig. 1) is an attractive development of these materials towards sustained drug delivery systems (DDS) applications.[4,5] Antibiotics[4] and anti-inflammatory drugs[5] have been evaluated for this purpose, and current studies are focusing on the class of antitumoral compounds. Techniques used include oscillatory rheometry, circular dichroism, Thioflavin T fluorescence, Fourier-transformed infrared (FT-IR) spectroscopy and transmission electron microscopy (TEM).

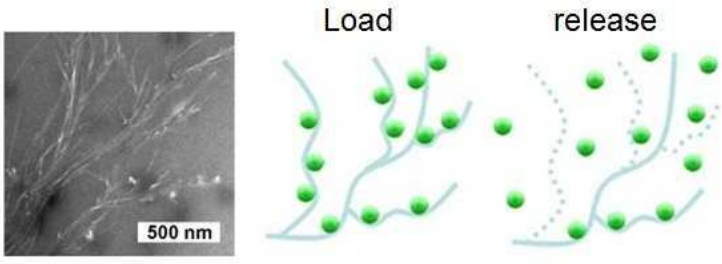

Fig.1. TEM image (left) and scheme (right) of a fibril-forming tripeptide (light blue lines) and drugs (green circles) assembled together for sustained drug release

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OPTIMIZATION AND CHEMICAL-PHYSICAL CHARACTERIZATION OF ASCn LIPOSOMES LOADED WITH KHELLIN

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Drug Delivery systems, as vesicles, represent a useful strategy to improve permeation, bioavailability and stability to oxidation of many natural substances. Ascorbyl octanoate (ASC8) and ascorbyl decanoate (ASC10) are useful antioxidant and amphiphilic molecules that can easily penetrate through the tissues [1]. The present study was conducted to formulate, optimize and then evaluate vesicles added with ascorbic acid derivatives (ASCn) and loaded with khellin to obtain nanocarriers with potential antioxidant and anti-vitiligo activity. Khellin is a furanochromone with a chemical structure closely resembling that of the psoralen family. If activated by UVA (365 nm), it can be used to stimulate inactive melanocytes in treatment of vitiligo, an acquired depigmention skin disorder affecting about 1% of the population [2]. In this paper the vesicles were prepared by Thin Layer Evaporation using 33 mg/ml of phosphatidylcholine, 12.1 mg/ml of ASC8 or 13.2 mg/ml of ASC10 and 0.5 mg/ml or 2 mg/ml of khellin. The resulting colloidal dispersions were stored in fridge at 4°C in order to estimate their physical-chemical stability for five weeks. The quantitative analysis were carried out using a HPLC1100-DAD. These nanocarriers were characterized for their size, polydispersity index (PDI), ζ potential, recovery, encapsulation efficiency (EE%), release and morphology. Moreover the vesicles were characterized in terms of Small-angle X-ray scattering (SAXS) and Differential Scanning Calorimetry (DSC) that were able to provide us with a general framework of the system's bilayer structure and organization as well as a release kinetics. Results showed that the vesicles had spherical shape and exhibit average sizes about 150 nm, the ζ potential varied from -45mV to -25 mV, the PDI was about 0.27 mV, EE% were between 75% and 90% while the recovery were between 90% and 100%. The drug release study was performed for 24 hours showed that after 9 hours all khellin were released from the vesicles. SAXS and DSC described the bilayer characteristics and the structural differences between loaded and empty systems as well as release kinetics. The findings suggest that these formulations can be useful to treat patients suffering from vitiligo.

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IN SITU GELLING SYSTEMS FOR THE LOCAL DELIVERY OF NATURAL COMPOUNDS IN THE TREATMENT OF ORAL MUCOSITIS

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Oral mucositis represents the more frequent and clinically significant complication of cyto-reductive chemotherapy and radiotherapy. This pathological condition refers to severe inflamed ulcerative lesions of the oral mucosa, associated to an intense pain [1].

The present work aims at developing *in situ* gelling systems to be loaded with an aqueous extract of *Hibiscus sabdariffa* (Hb extract), rich in polyphenols, for the treatment of oral mucositis; such systems should be characterized by an easy administration and by a prolonged permanence on the damaged tissue. To reach these targets, κ-carrageenan (κ-CAR) was selected as polymer able to gelify in presence of saliva ions, while hydroxypropylcellulose (HPC) was used as mucoadhesive agent. $CaCl_2$ was proposed as salt able to enhance the interaction between κ-CAR and saliva ions.

Different salt and polymer concentrations were considered in order to obtain a formulation having the following features: i) low viscosity at room temperature to facilitate the administration; ii) marked elastic properties at 37°C, functional to a protective action towards damaged tissues and iii) mucoadhesive properties. The most two promising systems, composed by κ-CAR (0.6% w/w (blank 1) or 0.4% w/w (blank 2)), HPC (1% w/w) and $CaCl_2$ (0.04% w/w), were loaded with the Hb extract, at two different concentrations, 0.2% (A) and 0.4% w/w (B). The extract loading did not alter the capability of the developed formulations to gelify in presence of saliva ions at 37°C and induced a decrease of viscosity values at 25°C, which was functional to an easy administration and to a good spreading on the damaged mucosa. Moreover, it was proved that loaded formulations, contrary to blanks, were characterized at 37°C by a prevalence of the elastic behavior on the viscous one, revealing a greater capacity to resist to the physiological removal mechanisms of the oral cavity. Both blank and loaded formulations showed good mucoadhesive properties, which guarantee a prolonged residence time of the systems and, thus, of the Hb extract on the oral mucosa.

Finally, *in vitro* studies, performed on human dermal fibroblasts, revealed a good biocompatibility and capability to support cell proliferation of both blank and loaded systems. The antioxidant potential of the formulations loaded with the Hb extract was also confirmed.

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ESCIN-BASED NANOVESICLES TO IMPROVE BERBERINE TOPICAL DELIVERY

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Berberine (BRB) is a natural isoquinoline alkaloid, used for many therapeutic activities by the Traditional Chinese Medicine, from the ancient times. Escin (ESN) is a mixture of triterpenic saponins that exhibits anti-inflammatory, anti-oedematous and venotonic properties and it was selected for both structural and functional activities [1]. Different liposomes, loaded with BRB HCl, were prepared according to the lipid film hydration method, changing the lipid mixture based on phosphatidylcholine, cholesterol and ESN. All liposomes showed optimal sizes, low polydispersity and spherical shape. The dialysis bag method, followed by HPLC-DAD analysis, was used to define the Encapsulation Efficiency (EE%) and to investigate the in vitro release of BRB HCl and ESN. EE% were about 67% for the first one and 94% for the second one. Concerning the kinetic release, it was found a maximum delivery around 75% and 25% respectively, within 24h. Liposomes had chemical-physical stability, until a month storage period, at 4°C. Deformability of the vesicles was evaluated by extrusion. Then, the formulations were tested by an ex vivo permeation assay using vertical diffusion Franz cells and rabbit ear skin. This system is useful to observe the distribution of BRB HCl into the various skin layers and to predict BRB HCl absorption, too, since it simulates the passive permeation thorough the full thickness skin. We compared rabbit ear skin with human abdomen skin by optical microscope and the anatomic structure was very similar in both tissues. According to these results, we chose rabbit ear skin as a suitable model in skin permeation studies for both lipophilic and hydrophilic permeants. By permeation studies, it can be observed that 6 h after the topical application of all liposomes, both molecules had not permeated the full thickness of the skin or were not quantified in the receptor chamber. However both BRB HCl and ESN were detectable after 24 hours. In order to validate the model, a slice of not treated skin was observed by optical microscope and no morphological alterations were found in the tissue. In conclusion, skin permeability was higher for BRB HCl loaded in ESN-based liposomes than BRB HCl loaded in the conventional liposomes, or respect to the free molecule. Therefore, these liposomes are deformable vesicles, able to cross the skin until the deepest layers of epidermis and suitable for dermatological applications to achieve a therapeutic synergy [2].

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Formulation development of a biopolymer-based multilayer film for the local treatment and wound repair of periodontitis

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The prolonged life and the recurrent use of new dental implant technologies suggest in the immediate future a strong demand for buccal dosage forms for the treatment of different dental health problems, such as treating post-implant surgery complications or chronic diseases like periodontitis and gingival retraction[1]. Therefore, our research project has been focused on the fabrication of a biopolymer-based multilayered film[2], which is bioadhesive and biocompatible with the unhealthy tissue and simultaneously releases the drug locally with a controlled rate.

In this study, films containing a binary mixture (70/30 %) of a film-forming polymer (like HPC, HPMC K750, HEC) and a biological product (like collagen peptide [MW 2-3 kDa], gelatin type A & B [Bloom strength 175 g] and an undisclosed novel product (NP) with potential anti-inflammatory activity) were prepared by solvent casting method. The dried films were evaluated to ensure optimum film features. Chlorhexidine digluconate as a model drug was eventually dosed into the films that showed the best characteristics. *In vitro* tests were conducted on a human monocytic cell line (THP1) to evaluate the toxicity and anti-inflammatory effect of the NP.

The mean weight and thickness of the films were found to be 53 ± 0.8 mg and 0.12 ± 0.1 mm, respectively. A study of surface morphology confirmed the homogeneity and smoothness of all the films. Moreover, the binary mixtures demonstrated statistically significant differences for *in vitro* mucoadhesion, mechanical, *ex vivo* residence time, swelling and erosion properties. A mixture containing HPC and NP was chosen as the best candidate for the biocompatible layer due to its higher work of adhesion and *ex vivo* residence time; whereas HPC was selected for the controlled release layer due to its low erosion index. The *in vitro* tests on the human monocytic cell line (THP1) showed evidence of good anti-inflammatory effect and no toxicity. Thus, these results allowed us to select suitable candidates for both the biocompatible and release-retarding layer for buccal application of film-forming polymers and future development of multilayer films.

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PHARMACEUTICAL DEVELOPMENT OF MESENCHYMAL EXTRACELLULAR VESICLE FORMULATIONS

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Background. Mesenchymal extracellular vesicles (EVs) represent a promising approach for treating several diseases, instead of their parental cells [1]. Unfortunately, a standardized, validated and GMP-compliant production process is still lacking, generating uncertainty about their biological effects [2]. The aim of this work is to develop a pilot production process for mesenchymal EVs, combining ultrafiltration (UF) with lyophilization (FD). **Methods.** To induce EV release, adipose-derived mesenchymal stem cells (MSCs) were cultured in serum-free medium for 24 hours. Supernatants were concentrated and purified by UF; cryoprotectant was added and the EV solution was lyophilized. Then freeze-dried (FD) product was characterized in terms of: i) total protein and lipid content, Nanoparticle Tracking Analysis (NTA), Fourier Transform Infrared Spectroscopy (FTIR), Thermogravimetric Analysis (TGA) and Differential Scanning Calorimetry (DSC); ii) safety (blood and cytocompatibility (on fibroblast and chondrocytes), at concentration of 0-150 mg/ml and 0-25 mg/ml, respectively); iii) potency (immunomodulation, evaluated as ability of FD secretome to suppress inflamed lymphocyte IFN-γ production compared to its parental cells). **Results.** Protein and lipid content, expressed as μg/mg of powder, were 59.7 ± 2.05 and 8.9 ± 0.14, respectively (mean values of 3 batches); NTA detected a vesicle mean diameter of 231.1 ± 10.2 nm, with a concentration of $9.02 \times 10^8 \pm 0.167 \times 10^7$ EVs per mg. The low-intensity bands in FTIR spectra around 1653 and 1547 cm^{-1} (amide I C=O stretching vibrations and amide II N-H banding vibrations, respectively) confirmed the presence of EVs. Absorbance bands at around 1457 and 1377 cm^{-1}, related to CH_2 and CH_3 groups, confirmed the presence of lipids and proteins. TGA analysis revealed a mass loss of 1.7% w/w due to water content, while DSC confirmed that the lyophilization process occurred without any interactions between the formulation components. FD secretome was not hematolytic at any of the tested concentrations, whereas it induced hemagglutination at 150 mg/ml. A dose-dependent reduction in cell metabolic activity was observed for all cell lines, even if it remained ≥ 60% also at 25 mg/ml. The amount of IFN-γ was reduced in a dose-dependent manner in inflamed lymphocyte incubated with MSCs or their secretome. At the highest dose tested, secretome was as effective as cells. **Conclusion.** These results support the pharmaceutical and clinical development of UF and FD mesenchymal EVs as new acellular Active Pharmaceutical Ingredient.

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PRODUCTION OF HOT MELT EXTRUDED POLY(VINYL ALCOHOL)-CIPROFLOXACIN FILAMENTS FOR FUSED DEPOSITION MODELING-3D-PRINTING TECHNOLOGY AND PERSONALIZED MEDICINES

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From a medical and therapeutic perspective, the fused deposition modeling by 3D-printing (FDM-3DP) is a pragmatic tool for producing personalized devices and drug dosage forms [1]. One of the main problems to deal with is the lack of availability of biocompatible and biodegradable polymers in form of filaments suitable for 3D-printing. Thus, the aim of this study is to investigate the potential of Hot Melt Extrusion for producing drug-loaded filaments with different drug concentrations, coupled with FDM-3DP technology for printing customized drug delivery systems.

Several batches of poly(vinyl alcohol) with different size distributions obtained by cryomilling (PVA degree of fineness: 4000-5000 μm, 1000-2000 μm, 600-1000 μm, 250-600 μm, less than 250 μm) and Ciprofloxacin were prepared varying polymer/drug ratio (from 10% w/w to 35% w/w of API) and then extruded as filaments with the single screw Noztek Touch Extruder. Digital modeling with CAD software Rhinoceros 5 and CAM software Ultimaker Cura 3.2.1 was used for the development of models and scaffolds for FDM-3DP. Moreover, printer's parameters (Ultimaker 3 FDM-3D printer) and operative specifics were refined for an optimal print of the lab-made drug-loaded filaments into the desired dosage forms. Scanning electron microscopy allowed observing the surface interaction between drug and PVA as well as the architecture of the drug-loaded filaments and of the printed products. Finally, the drug content and thermal behavior of mixtures, filaments and printlets were monitored.

The PVA particle size greatly affected the ability of the polymer to load the drug and to form with it homogeneous mixture together with the extrusion process. Coarse or moderately fine PVA powders showed better processability and reduced the drug loss during both the drug/polymer mixture and the extrusion processes. Finally, drug-loaded filaments with different drug concentrations were successfully printed.

These results can help to fill the gap given by the lack of biodegradable and printable drug filaments moving an important step towards a rapid manufacturing process for personalized galenic formulations.

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ORAL SPRAY CONGEALED MICROPARTICLES FOR THE LOCAL INTESTINAL DELIVERY OF BIOLOGICS

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During the past decades, therapeutic proteins and peptides have gained a wide attention due to the advantages of great selectivity, target-specificity and safety comparatively to the majority of small molecules drugs. However, oral administration of macromolecules is difficult because of the chemical instability in the gastrointestinal (GI) tract due to the harsh pH conditions of the stomach and the activity of the digestive peptidases [1]. Solid lipid microparticles (SLMs) are a promising carrier for oral delivery of protein drugs owing to their stability, biocompatibility and biodegradability. Due to the hydrophobic nature of the lipids, whereas almost all protein drugs are extremely hydrophilic, the production of SLMs by traditional methods allow poor and variable drug loading with very low encapsulation efficiency (E.E.) values, always below 50%. In addition, because of the labile nature of biologics, preparation techniques employing organic solvents should be avoided. *Spray congealing* is a solvent-free process and has showed to be a promising method for the encapsulation of biologics [2]. In the present research three different biologic molecules (β-galactosidase, catalase and glutathione) were encapsulated in spray congealed-SLMs in order to protect the active molecule from gastric environment and to achieve a local delivery to the intestine. Drug-loaded SLMs were characterized in terms of particle size, morphology, drug loading and E.E. value. The integrity of the encapsulated drug was assessed by means of different techniques with a particular focus on the ability to maintain the catalytic activity in case of therapeutic enzymes. The effectiveness of SLMs as drug delivery system for the oral administration of biologic drugs was evaluated simulating the pathway of the MPs through the GI tract. For this purpose, the different environments of the GI tract (e.g. gastric and intestinal fluids in fasted and fed conditions) were simulated using *biorelevant media*. Spray congealing ensured E.E. values close to 100% and no loss or degradation of the active drug during the preparation process. Results highlighted the influence of particle size and composition on the ability of the SLMs to protect the bioactive molecule from the degradation in gastric environment. In conclusion, the present study suggested the potential of spray congealed lipid-based systems to obtain a local delivery of a biologic drug by selecting appropriate lipid excipients and particle size.

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MALTODEXTRINS AS DRYING AUXILIARY AGENT FOR THE PREPARATION OF EASILY RESUSPENDABLE NANOPARTICLES

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To increase the long-term stability of poly(D,L-lactide-co-glycolide) nanoparticles (NP) a drying step is mandatory. Spray- and freeze-drying can induce their irreversible aggregation and, therefore, the use of a drying auxiliary agent is necessary. The aim of this work was to explore the ability of four grades of maltodextrins (MDX) as NP's auxiliary agent in spray- and freeze-drying.

PLGA NP were prepared by the solvent displacement method, using a polymer with a T_g of 36.5 °C. Compatibility was evaluated by DLS, after stirring NP with solutions of MDX (DE2, DE6, DE12 and DE38) at 2, 4 and 8% w/v concentrations using water or 0.9% NaCl as dispersants. Spray-drying process conditions were optimized by a Design of Experiment. Freeze-drying cycles were designed based on the thermal properties of MDX solutions. Dried products were reconstituted in water or 0.9% NaCl under gentle stirring and characterized by DLS after 5, 30 and 60 min.

NP had a monomodal size distribution with a size of about 160 nm and a Z-potential (ζ) of -31 mV. NP were compatible with all the aqueous solutions of MDX at all concentrations, even if DLS revealed a slight increment of NP size as a function of MDX grade and concentration. A concomitant increase of ζ was less evident for MDX DE2 and DE38 compared to DE6 and DE12, with values of about -26 and -24 mV, respectively. In 0.9% NaCl solution, only MDX DE2 and DE38 did not induce NP aggregation. These results appeared related to the organization of MDX onto NP surface, since both the ζ and the apparent persistence length of hydrated MDX [1] followed the same trend. Independently of the drying method, after reconstitution of the dried products, MDX DE2 was not effective in preserving NP features (size>400 nm). Conversely, MDX DE38 allowed an easy and complete reconstitution of NP. Its optimal concentrations resulted in the 2-4% w/v range. Indeed, monodisperse NP were obtained after 5 min of reconstitution independently of medium ionic strength (spray-dried product prepared by 2%w/v MDX DE38: 172±12 nm; freeze-dried product prepared by 4%w/v MDX DE38: 188±6 nm).

In conclusion, MDX DE38 appears an efficacious drying auxiliary agent for NP at concentrations lower than those reported in literature for polyols and sugars, independently of the drying process.

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THE EFFECT OF CHITOSAN COATING ON HUMAN SERUM ALBUMIN NANOPARTICLES FOR NOSE TO BRAIN DELIVERY

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The nose to brain pathway is emerging as new option for the delivery of therapeutics to the central nervous system. Recently, it was demonstrated that albumin nanoparticles (NPs), studied for intranasal administration, improved the delivery of tacrine and *(R)*-flurbiprofen [1, 2]. Based on these results, in this work was investigated the influence of chitosan (CS) coating on the performance of human serum albumin (HSA) NPs for nasal application. HSA is the most abundant plasma protein and possesses unique characteristics that make it an ideal candidate for the application in the field of nanotechnology. CS is a natural biodegradable polymer obtained by the deacetylation of chitin. The interaction between CS and HSA NPs causes the shift from negative to positive surface charge of HSA NPs. Positively charged NPs enhance drug internalization, as the result of ionic interactions with negatively charged cell membranes. Additionally, CS is capable of opening the tight junctions and it has mucoadhesive properties that allow a prolonging nasal residence time. HSA NPs were prepared by desolvation technique. CS coating was obtained adding chitosan solution to an equal volume of HSA NPs suspension under magnetic stirring. Dynamic Light Scattering analyses revealed that the mean particle sizes was 241±18 nm for HSA NPs and 261±8 nm for CS-HSA NPs. The ζ-potential was -47±3 for HSA NPs and +45±1 for CS-HSA NPs. Transmission electron microscopy observations confirmed the presence of CS on the surface of HSA NPs. The developed formulations showed excellent storage stability both as suspension and as freeze-dried product after 3 months. The mucoadhesion properties were assessed by means of the turbidimetric method. NPs were loaded with sulforhodamine B sodium salt as model drug and the effect of CS coating was investigated performing release studies by dialysis bag method, permeation and uptake experiments using Caco-2 and hCMEC/d3 cells as model of the respiratory epithelium and BBB (nasally administered drugs were also adsorbed into the circulation and they reach the brain if they are capable of crossing BBB), respectively. Furthermore, *in vitro* diffusion experiments were performed using rabbit nasal mucosa in a Franz-type permeation apparatus.

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IN VITRO AND IN VIVO STUDY OF A FLURBIPROFEN NASAL POWDER FOR ALZHEIMER'S DISEASE

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Purpose. To develop and characterize *in vitro* and *in vivo* a nasal powder suitable for nose-to-brain delivery of flurbiprofen as anti-inflammatory for early management of Alzheimer's disease.

Methods. Microparticles of flurbiprofen sodium were prepared by means of either the Nano Spray Dryer-90 and Mini Spray Dryer B-290 (Büchi) varying the feed solvent composition and the temperature to see the effect on yield of production and microparticle properties. Then, blends (50:50) of spray-dried drug microparticles with spray-dried excipient (mannitol-lecithin) microparticles were agglomerated by (1) vibration of the blend on a sieve stack (106 and 850 μm sieve's mesh size) or (2) tumbling of the blends in a 100-ml glass pan with deflected wall. Drug content (HPLC), particle size and size distribution (laser diffraction), solid state properties (DSC, PXRD), morphology (SEM) and *in vitro* drug transport across rabbit nasal mucosa (Franz cells, 4 h experiment) were evaluated for both microparticles and agglomerates. Plasma and brain pharmacokinetic of flurbiprofen in rats after nasal administration of Nano spray-dried flurbiprofen sodium microparticles (6 mg dose as flurbiprofen acid) and agglomerates thereof with excipient microparticles (3 mg dose as acid) were determined in comparison with intravenous (i.v.) administration of a drug's aqueous solution (4.5 mg as acid). UDS powder insufflator (Aptar) was employed as device for nasal powder delivery.

Results. The spray dryer equipment influenced the physico-chemical characteristics of the dried product and process yield. For the microparticles, smaller particle size and narrower size distribution as well as higher yields were obtained with the Nano spray drier. Agglomerates had a lower flurbiprofen sodium content (15-47%) compared to the theoretical value (50%) due to partial de-mixing of the blend during the agglomeration. The spray dryer also influenced the capability of microparticles to form the agglomerates, while the agglomeration method impacted on the yield. *In vitro* drug transport across rabbit nasal mucosa from was superimposable for the microparticles and the agglomerates (around 5 mg drug permeated per cm^2 tissue after 4 h). *In vivo*, UDS device delivered efficiently both nasal powders, but with higher performance when combined with the agglomerates (emitted powder: 66 ± 10% and 83 ± 4% for microparticles and agglomerates, respectively). The administration of nasal powders (microparticles and agglomerates) resulted into rapid drug absorption (plasma C_{max} within the first 10 min after administration) and enhanced drug transport to the brain compared to i.v. injection (1.2 ± 0.2 and 0.8 ± 0.2 μg flurbiprofen as acid/g tissue for microparticles and agglomerates, respectively; 0.63 ± 0.06 μg flurbiprofen as acid/g tissue for i.v. solution).

Conclusions. Agglomerates improved powder handling and dosing without affecting the *in vitro* biopharmaceutical behaviour of flurbiprofen compared to the primary drug microparticles. Combination of agglomerates containing flurbiprofen with a second active substance (insulin) in powder form for nasal administration is under investigation aiming a multi-target drug approach in Alzheimer's disease.

DEVELOPMENT AND TECHNOLOGICAL CHARACTERIZATION OF NOVEL FORMULATIONS FOR THE OCULAR DELIVERY OF CYCLOSPORINE A

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Cyclosporine A (CyA) is a cyclic undecapeptide with an immunosuppressive activity approved for the topical treatment of several immune-mediated ocular surface disorders such as the sever Dry Eye Syndrome (DES). Its high molecular weight, low water solubility and high lipophilicity makes the ocular bioavailability and the development of an ophthalmic formulation of CyA a real challenge. The only approved European marketed formulation for a "once a day" administration for treatment of DES is Ikervis®, a nanoemulsion containing 1mg/mL of CyA [1]. Nanoemulsions could present some disadvantages related to the stability, ocular irritation, and pharmacokinetics issues [2]. Aim of the present study was to evaluate a combined strategy comprising nanomicelles based on non-ionic surfactants and the addition of hyaluronic acid (HA) as mucoadhesive polymer to improve the ocular bioavailability of poorly soluble CyA. Different nanomicellar formulations were prepared changing the percentage of total surfactants (Vitamin E-TPGS:Octoxynol-40, 2.25:1) and HA. In this study, we selected full-factorial DOE to screen essential independent factors for outcomes CyA entrapment, size, and critical micellar concentration (CMC). The promising nanomicellar preparation, Nano1HA$_B$-CyA (0.1%w/w of CyA and 1%w/w of total surfactants), was evaluated in term of cytotoxicity, ocular biocompatibility (pH and osmolality), cloud point, regeneration time, and short chemical stability (until 3 months at 4 and 20 °C). To investigate the possible interaction among formulation components, an ATR-FTIR analysis was carried out on raw materials (CyA, HA, Vitamin E-TPGS, Octoxynol-40) and freeze-dried Nano1HA$_B$-CyA. *In vitro* drug release profile of Nano1HA$_B$-CyA was assessed at 32°C by using the dialysis method; an ethanolic solution of CyA (0.1%w/w) was used as reference. Moreover, *ex vivo* permeation studies were carried out on excised scleral tissue of New Zealand rabbit eyes; the ability of the nanomicelles both to permeate through and penetrate into the scleral barrier was determined; furthermore, the presence of fluorescein labelled nanomicelles (Nano1HA$_B$FITC-CyA) into the cornea and sclera was verified by histological analysis. Finally, the *in vivo* pharmacokinetic of the formulation in the tear fluid of New Zealand rabbits was evaluated by using appropriate references (CyA emulsion and nanomicelles without HA).

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Study of formulation and critical compression parameters of Ibuprofen/Sucralfate three-layer tablets

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Purpose:

Multi-layer tablets (MLT) are designed for the manufacturing of fixed-dose combination products that simplify the medication regimen and potentially increase the patient's compliance. MLT are heterogeneous systems in which two layers of compacted powders are separated between them by a discrete interface. The hardness and the delamination tendency of MLT depend not only on the layer composition but also on the deformation property of each layer during tableting.

Methods:

Tablets were made with different sucralfate formulations and, by using a compression simulator, it was possible to study the critical parameters involved in the compression process. Based on an experimental design, ten formulations for the two layers of sucralfate have been manufactured, which differ for the percentage of mycrocristalline cellulose, partially pregelatinized starch and lactose but not in the content of the active ingredient (ibuprofen). TLT were manufactured using a Styl'One Evolution Rotary Tablet Press Simulator (Medelpharm, France). This apparatus is a single punch tableting press, in which the displacements of the lower and upper punches are electronically controlled. The tablets were produced using oblong Euro D punches (17.50 x 8.50 mm), at different pre-compression force (0, 2 and 4 kN) and compression forces (10, 20 and 30 kN). The compaction process was performed using Advanced Analysis Software.

Results:

From the analysis of critical compression parameters, for all sucralfate formulations the plastic energy raised as the applied compression force increased. The TLT with the sucralfate layers containing pregelatinized starch, elastic material, showed higher ejection energy, compared to the formulations with microcrystalline cellulose, plastic material, and/or lactose, brittle material. In presence of pregelatinized starch, when the force exerted from the upper punch is removed in the phase of TLT ejection from the die, the friction radial forces increased since the tablet tends to expand in the die and then the ejection energy augmented. Moreover, the sucralfate layers containing microcrystalline cellulose, exhibited a greater tendency to layer separation, due to a reduced interfacial strength between sucralfate and ibuprofen layers. The addition of lactose to the sucralfate formulation was beneficial for the layer adhesion during the manufacturing of the TLT.

Conclusions:

The presence of microcrystalline cellulose, lactose and pregelatinized starch in the sucralfate layers can affect, in different manner, the critical parameters of the manufacturing of ibuprofen/sucralfate three-layer tablets.

A LIPOSOMAL FORMULATION FOR INFLAMMATION TARGETING

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Inflammation is a pathological condition leading to different diseases including, among others, atherosclerosis, neurodegenerative diseases and cancer [1, 2, 3, 4]. Glioblastoma is the most frequent brain tumor (69% of all incident cases of astrocytic and oligodendroglial tumors) [5]. Glioblastoma cells overexpress VCAM-1 (vascular cell adhesion molecule 1), a membrane protein considered an inflammation marker, being involved in the process of leukocyte transmigration across activated endothelial walls.

We developed a new liposomal system targeting VCAM-1 through a three-step pretargeting involving NAMP, a previously synthesized biotin derivative linked to a VCAM-1 binding peptide. This molecule has been conceived to be administered parenterally, followed by injection of avidin, to exploit the biotin-avidin high affinity complex; the pretargeting will be achieved by final administration of biotinylated liposomes.

Biotinylated stealth liposomes were prepared by the thin film hydration method. After extrusion through polycarbonate filters, we determined the size of liposomes using PCS (photon correlation spectroscopy) and their zeta potential through electrophoretic mobility by laser Doppler micro-electrophoresis. The liposomal suspension was purified by gel filtration on a Sephadex G-50 column.

The same formulation including CM-DiI fluorescent dye was tested *in vitro* on human glioblastoma cell cultures isolated from postsurgical specimens, applying the three-step protocol; control cells were treated only with biotinylated liposomes. By live confocal fluorescence microscopy, we observed a preferential interaction of the biotinylated liposomes with the membranes of those cells previously treated with NAMP and avidin.

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PRODUCTION AND IDENTIFICATION OF MANNITOL POLYMORPHS FOR USE IN DPI FORMULATION

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The aim of this study was to obtain different polymorphs of mannitol in kinetically stable form and to investigate their solid-state and physico-chemical properties in view of its use in dry powder inhalers as a carrier alternative to lactose.

The hydrate and β forms were produced by freeze-drying a solution of either Pearlitol® 200SD or Pearlitol® 160C in deionized water at the concentrations of 4 % or 18 % w/v. The α form was prepared by seeding and rapid cooling of a Pearlitol® 200SD (0.8% w/v) solution in methanol in presence of poly (vinyl alcohol) (PVA) (from 0.5 to 2 % w/w of mannitol). The δ form was obtained by modifying the method proposed by Cares-Pacheco et al. [1] and Vanhoorne et al. [2], namely, by crystallization of an aqueous mannitol solution (18% w/v) in presence of PVP K30 at from 0.5 to 4% w/w of mannitol using acetone as antisolvent.

The solid state and physical characteristics of the mannitol solid phases were assessed by X-Ray diffraction on powders, differential scanning calorimetry (DSC), scanning electron microscopy and hot stage microscopy.

The obtained mannitol solid phases were unambiguously identified by comparing their X-Ray diffraction patterns with those obtained from the Cambridge Crystallographic Database.

The hydrate form of mannitol was obtained by freeze-drying with lower drying compared to the production technique of β form. However, this form proved to be not enough kinetically stable and rapidly converted into the stable β form. The possibility to obtain pure and kinetically stable δ form was investigated by changing the concentration of PVP in the mannitol solution in order to minimize the polymer concentration. The best results were obtained with 1% PVP. The kinetically stable α form was obtained with 2% PVA. The DSC analysis afforded melting peaks for δ mannitol, α and β mannitol at the onset temperature of 155.4 ± 0.4 ^{o}C, 164.4 ± 0.1 ^{o}C and 165.2 ± 0.5 ^{o}C respectively.

We concluded that pure metastable polymorphs of mannitol can be obtained in kinetically stable form by adding small amounts of stabilizing polymers such as PVA and PVP. The isolation of pure mannitol crystal phases is the starting point and the condition *sine qua non* to prepare DPIs with different mannitol polymorphs as carrier and to investigate the effect of mannitol solid state properties on drug aerosolization performances.

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MANNOSE-TARGETED CATIONIC GLYCOPOLYMERS AS NEW TOOL FOR OLIGONUCLEOTIDES-BASED IMMUNOTHERAPY

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Tumor immunology is changing the landscape of modern medicine and anticancer therapy, ranging from tumor antigenic peptides to nucleic acids treatments. In particular, Reversible Addition Fragmentation chain Transfer (RAFT) polymerization [1] was exploited here for the synthesis of a small library of diblock copolymers aimed to deliver oligonucleotides (ONs) to dendritic cells and to trigger the immune response and memory against cancer. These novel materials were designed with a poly-cationic agmatine acrylamide block to condense ONs and with a glycopolymer block to actively and selectively target Mannose Receptor (MR) on immune cells [2]. Importantly, the system will also provide nucleic acid protection against fast degradation, minimizing its interactions with extracellular nucleases.

Three cationic block copolymers (Man_{15}-*b*-Agm_{12}, Man_{29}-*b*-Agm_{25} and Man_{59}-*b*-Agm_{45}) were obtained by fast RAFT polymerization using Agmatine acrylamide (Agm) and D-Mannose acrylate (Man) as monomers. Polymers were generated with same ratio of monomers and increasing length to select the one that ensure suitable loading, stability, delivery. GPC analysis on glycopolymers confirmed their narrow molecular weight distribution, with a polydispersity of 1.13, 1.43 and 1.29, respectively. Glycopolyplexes (GPPs) were obtained incubating each block co-polymer with a 19-base ssDNA, used as model oligonucleotide. The optimum N/P ratio to achieve complete ssDNA complexation was evaluated by gel electrophoresis. A complete DNA complexation occurred also in presence of physiological concentration of heparin at N/P ratios higher than 10, 5 and 3 for Man_{15}-*b*-Agm_{12}, Man_{29}-*b*-Agm_{25} and Man_{59}-*b*-Agm_{45}, respectively. Furthermore, DLS and TEM characterization confirmed the formation of GPPs with narrow size distribution in the range of 25-45 nm, depending on the polymer size. Finally, flow cytometric studies revealed a remarkably high and specific recognition by mannose receptor expressing cells (CHO-MR) for Man_{15}-*b*-Agm_{12} and Man_{29}-*b*-Agm_{25} polyplexes with negligible internalization by cells that do not express the receptor (CHO). Man_{58}-*b*-Agm_{45} GPPs showed 2-folds higher internalization in CHO-MR cells as compared to control cells.

Confocal studies are ongoing to investigate cell uptake and endosomal escape of the GPPs thus favoring expression of antigens.

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NOVEL ALBUMIN-BASED NANOPARTICLES AS NEW PLATFORM FOR CANCER DRUG DELIVERY

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Resistance to chemotherapy is a major problem that limits the effectiveness of a successful treatment of cancer. Tumors may be intrinsically resistant to one or more anticancer drugs or be initially sensitive to them and acquire the resistance after repeated treatments. The anthracycline doxorubicin (DOXO) is an antineoplastic agent used in the treatment of a wide range of cancers, such as multiple myeloma, lung, ovarian, gastric, thyroid, breast, sarcoma, and pediatric cancer. In addition to cardiovascular side effects, DOXO can also cause resistance, in a mechanism that involves cellular transporters. For this reason, new approaches to overcome DOXO resistance has been the focus of extensive research. Albumin-based nanoparticles represent an ideal nanocarrier system because of its drug-loading capacity, biocompatibility, high availability, easy purification mechanism and low cost. Besides, this advanced drug delivery system can interact with both hydrophobic and hydrophilic therapeutic molecules, provide controlled release of drug and be easy modified due to the presence of functionally charged surface groups.

In this work, DOXO-loaded bovine serum albumin-based nanoparticles were prepared by a coacervation method. Glycol chitosan coated (GC DOXO-NP) and un-coated (DOXO-NP) formulations were prepared and compared. The formulations were characterized measuring the size, the polydispersity index and the zeta potential by laser light scattering. Transmission electron microscopy (TEM) was used for studying the nanoparticles morphology. The formulation stability was confirmed in short and long term. All the formulations showed a size of about 300 nm. DOXO was encapsulated in a great extent and was released from the nanoparticles with a prolonged *in vitro* release kinetics. Biological assays were performed on A2780 res, an ovarian cancer cell line resistance for DOXO. Cell viability assay (MTT test) was carried out and the nanoparticles showed an higher cytotoxicity than the free drug after 24 and 48 hours of incubation. Moreover, clonogenic assay showed that GC DOXO-NP and DOXO-NP reduced in a remarkable manner the proliferation of cells. In conclusion, the cell uptake of the different DOXO-NP formulations was evaluated by confocal microscopy.

Resistance to chemotherapy limits the effectiveness of anti-cancer drug treatment. Since DOXO-NPs showed a significant cytotoxicity on a cell line resistance for DOXO, they might represent a novel approach to overcome it and improve the efficacy of cancer therapy.

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PREPARATION, CHARACTERIZATION AND OPTIMIZATION OF BERBERINE LOADED PLGA-PEG NANOPARTICLES BY RESPONSE SURFACE METHODOLOGY.

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Nanoprecipitation is a straightforward, rapid and easy to perform technique which allows the production of small and homogeneous nanoparticles (NPs) in which a wide range of preformed polymers can be used [1]. This technique is mostly suitable for hydrophobic compounds thus the encapsulation of hydrophilic molecules is limited. Berberine chloride (BBR, selected as hydrophilic drug) is a quaternary ammonium salt isolated from a variety of Chinese herbs, which exhibits many pharmacological actions such as anti-tumor effect [2]. Although BBR has many beneficial properties there are certain limitations in its clinical applications, such as its poor bioavailability and several risk of adverse reactions, thus nanoformulations of BBR could help in enhancing its use in the pharmaceutical field [3]. During development of nanoformulations, different variables in the process can affect the properties of NPs. Testing numerous factors to obtain the best formulation condition is tedious, time consuming and often leads to confounding results. The design of experiments is a very useful tool for finding the best experimental condition, reducing the number of experiments and providing a reliable interpretation of the results [4]. The present work focuses on critical parameters of the nanoprecipitation procedure to obtain PLGA-PEG NPs having suitable physico-chemical properties to encapsulate BBR. For this purpose, with the help of D-Optimal Response Surface Methodology (RMS) design the polymer concentration and the organic to aqueous phase ratio were studied at 3 different levels (independent variables) on NPs size (Z-Ave), polydispersity (PDI) and zeta potential (ZP) (dependent variables). The optimized NPs loaded with BBR, were homogenous with mean size less than 200 nm, a negative surface charge (similar values compared to the unloaded NPs) and showed a good encapsulation efficiency. BBR was found in an amorphous phase within the NPs system as revealed by thermal analysis. Considering the good correlation between observed and predicted results we can claim that RSM is a successful tool to design new pharmaceutical formulations with the least number of experiments and to interpret experimental data.

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Red grape pomace extract loaded in soluble fibre-enriched liposomes as prebiotic and antioxidant system for intestinal protection

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In this work, we combined the antioxidant effect of grape pomace extract (in ethanol/propylene glycol) with the prebiotic effect of a soluble fibre in phospholipid vesicles [1]. The soluble fibre Nutriose FM06 was used at different concentrations (20, 40 and 60% w/v), and liposomes without fiber were used as a reference. The aim of the study was to obtain grape-loaded soluble fibre-enriched liposomes, namely grape nutriosomes, and test their efficacy on intestinal protection [2]. The vesicles were prepared by direct sonication and were fully characterized observing the morphology and structure by cryo-TEM, estimating size, zeta potential by a Zetasizer nano, and the entrapment efficiency by measuring the antioxidant activity of the dispersions with the DPPH assay. The average diameter of nutriosomes was around 150 nm, with polydispersity index around 0.13, and highly negative zeta potential (~-79±7 mV). The entrapment efficiency was around 88±11%. The vesicles were stable, as the size and zeta potential remained unchanged during six months of storage at 25°C.

Size, zeta potential and entrapment efficiency were also measured in acidic (pH 2) and basic (pH 7) environment after 2 and 6 h, respectively, to determine both the stability and the ability of the formulations to retain the extract. After 2 h at pH 2, the mean diameter of nutriosomes increased up to 496±121 nm, and they were able to retain 84±7% of the incorporated extract. After 6 h at pH 7, the mean diameter of nutriosomes was unchanged, but they slowly released the incorporated extract (59±9%). The results disclosed the ability of nutriosomes to protect the extract from the acidic environment of the stomach, favouring the controlled release in the intestine were the extract and the pre-biotic fiber are expected to exert their beneficial activities.neficial efficacy of the grape nutriosomes was tested against oxidative stress induced by hydrogen peroxide in Caco2 cells. All the formulations tested showed high biocompatibility and protective effect against hydrogen peroxide damage. Overall results confirmed the suitable application of grape nutriosomes in intestinal protection.

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CAN MICROFLUIDIC TECHNIQUE ENHANCE CD44-TARGETED HYALURONIC ACID DECORATED NANOSYSTEMS PERFORMANCES ?

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Microfluidics is a new and emerging science and technology field, its first major application in nanomedicine was the improvement of the quality of NPs synthesis through the control of the reaction environment. Microfluidic reactors enable rapid mixing of reagents, control of temperature, and precise spatiotemporal manipulation of reactions that are difficult, if not impossible, in larger reactors [1]. In microfluidic NP synthesis, physicochemical properties of NPs, which are strictly correlate to their *in vitro/ in vivo* fate, could be precisely controlled in a reproducible manner [2].

The aim of this work is to investigate the *in vitro* performance of hyaluronic acid / chitosan based nanogels (HA/CS NPs) and how the preparation technique could improve the nanosystems *in vitro* behavior. HA should permit a CD44 targeting because it is the natural ligand for CD44 that is significantly expressed in various cellular population as mesenchymal and cancer cells. NPs were loaded with Everolimus (EVE), drug widely used in cancer therapy because of its ability to reduce cell proliferation. NPs were prepared using NanoAssemblr^{TM} platform composed by a microfluidic device with a microchannel characterized by a staggered herringbone structure. HA/CS NPs presented mean size of 159.00 ± 7.28 nm (PDI= 0.306 ± 0.053) and negative surface charge (- 20.85 ± 3.16 mV). TEM analysis revealed spherical NPs. EVE encapsulation was of 88.12 ± 20.76 μg EVE/mg NPs (EE%=54.56 ± 7.45). Cell viability, evaluated by MTT assay, confirmed the excellent biocompatibility of the placebo NPs. Anti-proliferative effect of EVE loaded NPs was tested by bromodeossiuridine assay on two different cell types: mesenchymal cells from ovarian follicle and mesenchymal cells from bone marrow. Both were characterized as CD44 overexpressing cells. HA/CS NPs and cells were incubated together for 30 min, 1.5 h and 4h. Untreated cells, EVE solution and placebo HA/CS NPs were used as control. EVE effect resulted to be cell line dependent nevertheless an important decrease in proliferation comparable to EVE solution was observed after 4 hours of incubation with EVE loaded NPs, when cell proliferation was about 1%, for all cell line. NPs uptake studies are still in progress.

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SYNTHESIS AND CHARACTERIZATION OF METHACRYLATED CHONDROITIN SULFATE AS A PROMISING MATERIAL FOR BIOMEDICAL APPLICATIONS

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In this work, chondroitin-4-sulphate (CS) was used to prepare novel chemical hydrogels and implantable scaffolds with potential application in biomedical and pharmaceutical fields. CS is a glycosaminoglycan found in articular cartilage and connective tissue which contributes to maintain the structural integrity of cartilage and improves tissue regeneration. These features make CS an ideal candidate to fabricate novel biomaterials for tissue engineering applications [1]. The aim of the study was to synthesize and characterize methacrylated CS (CS-MA) able to form chemical hydrogels as suitable scaffolds for cartilage regeneration and protein delivery. CS-MA was synthesized reacting a CS-tetrabuthylammonium salt (CS:TBA) with glycidyl methacrylate in the presence of 4(N,N-dimethylamino)pyridine (4-DMAP) [2]. The reaction was carried out under different experimental conditions changing the molar ratio among the reagents and the exchange degree of CS:TBA to investigate the effect of these parameters on the derivatization degree (DD%) of CS-MA. The obtained derivatives were characterized by FT-IR, ^{1}H- and ^{13}C-NMR. FT-IR spectra evidenced the successful incorporation of methacrylate groups, which was further confirmed by the presence of the signals corresponding to the vinyl protons of MA in ^{1}H-NMR spectra. ^{1}H-NMR was also used to calculate the DD% of CS-MA employing nicotinamide as internal standard. A linear relationship was found between the molar ratio of the reagents used and the obtained DD%, which can be finely tuned by opportunely setting the reaction conditions. ^{1}H- and ^{13}C-NMR spectra also reveal that the reaction proceed via a double mechanism: transesterification and epoxide ring-opening [3]. So, a deeper structural characterization of CS-MA was carried out using HSQC, HMBC and COSY to confirm the double mechanism and to investigate if the reaction occurs on both primary and secondary OH groups of CS.

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AEROGEL CAPSULES LOADED WITH KETOPROFEN LYSINATE FOR DIRECT ADMINISTRATION ON WOUNDS

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The disadvantages of traditional topical formulations, as short residence times, the impossibility to provide an adequate environment at the wound bed and the production of peritoneal trauma when removed are driving the search for advanced formulations that tackle this problems, specially when it comes to the healing of acute or chronic wounds [1, 2]. Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most frequently medications used worldwide and, in the case of pain management, its use is associated with a lower presence of inflammatory cytokines thus contributing to a more favorable inflammatory response, leading to successful wound healing, but the bioavailability of such kind of drugs has been compromised due to the lower dissolution rate [3]. For this reason, in the present work we proposed a new topical formulation based on a highly porous polysaccharide-based matrix able to absorb a great amount of exudates from wounds and able to control the release NSAIDs directly to the wound site by controlling gel properties. Gel capsules, formed by an alginate shell and an o/w/o emulsion core containing 5%, 10% and 20% (w/w) of ketoprofen lysinate (KL), solubilized in the aqueous phase of the emulsion, were manufactured by prilling technique in co-axial mode. Supercritical drying was carried out in an autoclave at 40ºC and at 125 bar using a constant flow of CO_2 for the removal of the oily phase from the gel capsules thus obtaining the corresponding aerogel capsules with very low densities and a high porosity. Textural properties of aerogel capsules were studied: values of specific surface area, pore volume and pore density were in the expected range for this kind of materials, that are presented as an alternative solution to the drawbacks of traditional topical formulations.

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UP-CICLYING OF HAZELNUT BY PRODUCT IN A NEW FUNCTIONAL SPRAY DRIED POWDER

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A large volume of waste and by-products are generated by agro-industrial processing. In recent years, scientific research has shown greater interest in the recovery of their functional and nutritional components, improving them from waste to bioactive resources for health or food products. Hazelnut (*Corylus avellana* L.) skins and shells are the main by-products of the kernel industry processing [1]. In a previous work, a shell polar extract (HSE) was produced and its chemical investigation led to the isolation and characterization of different classes of phenolic compounds, including neolignans, and a diarylheptanoid, which contribute to a high total polyphenol content expressed as gallic acid equivalents. HSE showed a significant scavenging activity *in vitro* against the radical DPPH and an inhibitory effect on human melanoma and cervical cell lines, inducing apoptosis by caspase-3 activation [2]. Technological procedures can convert the dried extracts into higher-value, concentrated and easily transportable products with a long-lasting shelf life [3]. Thus, in this work, a spray drying powder loading HSE, was produced. HSE was encapsulated in a tandem polymeric matrix based on L-proline (P) as loading carrier, Hydroxyethylcellulose (HEC) and Pectin as additional coating co-polymers. Moreover, in order to improve the solubility of HSE raw material, lecithin (L) and ethanol were used in the feed suspension. The polymeric phase concentration selected and the hot-cold-hot method used led to satisfactory results, showing a high loading efficiency (LE) (95%). Particles resulted well formed and free of fractures with unaltered antiradical activity, and an improved *in vitro* water dissolution profile. The thermal analysis confirmed that HSE well interacts with the polymeric matrix in the particles formation. The developed method seems to be suitable to transform HSE raw material in a stable powder to be enclosed in a topical or oral dosage form as chemopreventive and antioxidant ingredient.

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BRIJ® NIOSOMES AND VEGETAL OIL NANOEMULSIONS FOR TOPICAL DELIVERY: A COMPARATIVE STUDY

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The aim of the present work is the comparison between different nanocarriers, such as nanoemulsions (NEs) and niosomes by Brij®72 (Polyoxyethylene Stearyl Ether), for the topical delivery of active compounds. Brij®72 seems to be able to increase drug residence time in the stratum corneum (SC), limiting transdermal permeation [1]. In order to evaluate the drug loading capacity of both formulations, a hydrophobic model drug was used. All formulations were extensively characterized in terms of size, ζ-potential and drug entrapment efficiency (EE %) [2, 3]. Furthermore, stability studies were carried out over time at two different storage temperatures (4°C and 25°C). The shape and the surface morphology of all samples were also investigated using Atomic Force Microscopy (AFM) and Small-Angle X-ray Scattering (SAXS) [4]. Differences in terms of fluidity, microviscosity and polarity of the non-polar phase between niosomes and nanoemulsions were investigated.

From the obtained results, all formulations have suitable dimensions for topical delivery [5], but NEs show a higher drug EE% than niosomes. Further studies will be carried out in order to confirm that NEs could be a promising drug delivery systems for the treatment of skin diseases.

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OLANZAPINE ORODISPERSIBLE FILMS: A FEASIBILITY STUDY

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Abstract

Olanzapine (OLZ) is an effective atypical antipsychotic drug with an acceptable safety profile widely used in the treatment of psychiatric disorders. The administration as orodispersible tablets meets the patients' preferences improving the medication adherence [1]. However, this drug presents some formulative issues due to the conversion into different polymorphic and pseudopolymorphic forms as a function of the preparation process and in presence of water [2]. This feature is particularly relevant in the design OLZ loaded orodispersible films (ODF) which represent a valuable alternative to orodispersible tables [3]. ODF are generally manufactured by hot-melt extrusion or solvent-casting.

In this preliminary study, the feasibility to prepare OLZ loaded ODF by a water-based process was investigated. In particular, ODF were obtained by laminating and casting an aqueous dispersion of OLZ and maltodextrins (MDX) plasticized with glycerol and dried by a solvent casting technique in order to obtain the theorical drug content of 10 mg per 6 cm^2 ODF. ODF were characterized in terms of disintegration time and dissolution profile in deionized water and phosphate buffer pH 6. The solid-state of OLZ loaded ODF was studied using X-ray diffraction (XRP) and ATR-FTIR spectroscopy. ODF visually appeared homogeneous with smooth surface and yellow colored due to the presence of olanzapine. They were easy-to-handle and easy-to-cut into desired dimensions without cracks. The film thickness was about 140±4.5 μm. The disintegration time was about 40 s, complying the requirements of Ph. Eur. monograph. An erratic drug release pattern was observed during the *in vitro* dissolution of ODF with a concomitant formation of a yellow precipitate after 3 min. XRD patterns of OLZ loaded into ODF evidenced the phase conversion from anhydrous form into dihydrate one. This feature was in line with a variation in ATR-FTIR spectra since a shift in the stretching bands of CN group of OLZ loaded in films was evident with respect the pure drug.

Overall data suggested that OLZ undergoes to phase transition after solvent casting compromising the biopharmaceutical performances due to its variation in solubility. Hence, a solvent-free preparation process or different excipients must be evaluated to avoid the variation in OLZ solid-state.

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FROM VEGETABLE OILS TO SOLID MICROPARTICLES BY PRILLING TECHNOLOGY

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From the 50s to today a continuous and growing development of microencapsulation technology has taken place. The prilling is one of the most important methods for the production of innovative pharmaceutical forms, now widely used in a large number of industrial sectors: food, agricultural, textile, cosmetic and pharmaceutical. The use of particle systems allows to modify and improve the morphological-structural characteristics and the chemical-physical properties of a substance. Prilling allows to obtain homogeneous microcapsules in terms of size, characterized by high encapsulation efficiency [1]. Prilling technique is based on breaking a laminar flow of a liquid, which is separated into single-dimensional drops by a mechanical vibration applied to a nozzle where a solution or emulsion comes out [2]. The aim of this study is the production of solid alginate (ALG) based poli-nucleate microparticles from vegetable oils using the prilling technology which in turn by using a drying process are giving pellets as solid dosage forms of oils.

For this purpose, we selected vegetable oil with high concentration of fatty acids and phytosterols that possess a marked anti-inflammatory and antiandrogenic activity, potentially efficient in the management of benign prostatic hyperplasia (BPH). The microcapsules were characterized in terms of yield, size, efficiency of encapsulation, and swelling studies. The encapsulation of the oil makes it possible to mask its taste and smell, to increase its stability, obtaining a product with a high total fatty acid content compared to those present on the market today.

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Design and application of film-coatings for colon delivery of drugs based on a combined formulation approach

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Introduction: my doctoral research project is focused on the design of an oral colon delivery system based on a combined formulation strategy, which would be intended to overcome the limitations of approaches relying on single physiological characteristics of the intestine and affected by the relevant intra- and inter-subject variability. The system proposed comprises a drug core, an inner swellable/erodible polymer layer and an outer film composed of blended enteric soluble polymer and microbially-degraded polysaccharide acting as a colon-selective pore-former. In this part of the work, the use of various naturally-occurring polysaccharides as potential colon degradable excipients was explored.

Methods: aqueous or hydro-alcoholic coating dispersions were based on Eudragit®S in admixture with high-amylose starch (Amylo N460), pectin (Aglupectin HS-RP), chondroitin sulphate sodium (ScanDroitin) and/or chitosan (7:3 solid weight ratio). Triethyl citrate and glyceryl monostearate were employed as a plasticizer and an anti-tacking agent. Minitablets (4mm), as such or coated with hydroxypropyl methylcellulose (HPMC, Methocel®E50, 100-270μm coat thickness), "0" hard-gelatin capsules and hydroxypropyl cellulose (HPC, Klucel™LF) molded capsular devices (600μm shell thickness), all containing acetaminophen as a drug tracer (30mg), were used as cores. Minitablets were coated by fluid bed, whereas gelatin as well as HPC capsules were pan-coated. Coated systems were oven-cured at 35° or 40°C for 24, 48 or 72h. Release tests (n=3) were carried out at t=0 and after storage under ambient or 40±2°C/75±5 RH conditions by USP39 apparatus 2 in 0.1N HCl for 2 h and then phosphate buffer pH 7.4 (800mL) at 37±0.5°C and 100rpm (UV assay at 248nm).

Results: film-coating of minitablets was successfully performed with each Eudragit®S mixture with the investigated polysaccharides. In the case of the coating system containing Amylo N460, the process was also carried out using gelatin and HPC capsules as cores, after adjustment of operating and formulation parameters. The applied films showed gastroresistance properties depending on thickness, except for the chondroitin sulphate-containing one. After pH change, pulsatile release profiles were obtained, with more extended lag phases from systems based on HPMC-coated minitablet or HPC capsule cores. The performance of Amylo N460-containing systems turned out consistent when release studies were repeated after storage both at ambient and accelerated conditions.

Conclusion: all investigated polysaccharides were proved suitable for incorporation into Eudragit®S films, which were applied to different substrates by spray-coating leading to the desired release pattern. Physical stability studies are ongoing for each formulation obtained, and the relevant release performance will also be investigated by the use of simulated colonic fluid of proper composition in order to evaluate the role of microbiota.

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PREPARATION AND CHARACTERIZATION OF SILK SERICIN NANOSYSTEMS FOR PHARMACEUTICAL APPLICATIONS

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Background. Silk sericin (SS), a glue-like and hydrophilic protein, was considered for many years a waste product in textile industry. Recently, SS was proposed and studied for pharmaceutical applications thanks to its biological properties, biocompatibility, biodegradability and non-immunogenicity; in drug delivery field, SS can be used as a biopolymer for nanostructured device formulation [1]. The aim of this work is to develop self-assembled SS-based nanosystems able to load both of hydrophilic and hydrophobic drugs, without the use of cytotoxic crosslinking agents. **Methods.** Sericin nanoparticles (SNPs) were obtained by self-assembly technique, using poloxamer (P) as stabilizer [2]. SNPs had been loaded with two model drugs: curcumin (C), a hydrophobic compound with different biological properties, and rhodamine B (R), a hydrophilic model molecule. SS, P and selected drug (at the concentration of 0.05 and 0.5 mg/ml for C; 0.05 mg/ml for R) were dispersed in DMSO; obtained suspension was added to aqueous solution under magnetic stirring. SNPs suspensions were dialyzed against water (12-14 kDa) to remove solvent and unreacted compounds, and then freeze-dried. SNPs were finally characterized in terms of drug loading, particle size distribution (nanoparticle tracking analysis), morphology (SEM), and physico-chemical properties (FTIR, DSC). **Results.** C loading was positively correlated to the drug concentration used during the SNPs preparation, ranging between 0.01 and 0.6 % w/w; the R loading was 0.002 % w/w. SNPs mean particle size distribution was 137.08±36.64 nm, without differences between loaded and unloaded nanosystems. SEM images demonstrated a partially aggregation of SNPs, which appeared rounded and pretty regular shape. SS and P presence in SNPs was confirmed by FTIR and DSC. FTIR spectra presented SS peaks of amide I, amide II and amide III at 1657, 1537 and 1200 cm^{-1}, respectively. P peaks were in the region of non-ionic surfactants at about 1100 cm^{-1}, at 3200-3500 cm^{-1} and at 2820-2980 cm^{-1}. For loaded SNPs, IR spectra did not detect the presence of rhodamine, probably due to the very low drug content, while showed a peak at 1513 cm^{-1} which could be attributed at the presence of the curcumin. Thermal analysis showed two endothermic peaks: one of poloxamer fusion (56.28°C, ΔHm= 118.2 J g^{-1}) and a SS decomposition peak (390°C, ΔHm= 296 J g^{-1}). **Conclusions.** We demonstrated a good correlation in SNPs particle size distribution, and morphology; whereas improvement on drug loading technique are to be further investigated to achieve an effective drug delivery carrier for pharmaceutical applications.

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MECHNICAL PROPRIETIES OF PRESS-COATED TABLETS : EFFECT OF THE INNER CORE

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In the last few years, the use of press coating technique has achieved huge increase for the formulation of modified release systems such as chronotherapeutic and colon targeting drug delivery systems [1]. The press coating technique is a simple technology used to provide tablets with a programmable lag phase, followed by a fast or sustained drug release after administration. The immediate-release core is coated with a layer of polymeric material that can dissolve, erode or swell in an aqueous environment.
The information currently available on the press coating tablets is mostly on the drug release kinetic of the formulations, while there are no information about the influence of the characteristics of the inner core on the mechanical properties of the final tablet.
This study analyse the effect of the inner core mechanical and rheological properties on the press-coated tablets, taking into consideration the compaction mechanism, the plasticity of the materials and the porosity of the cores. For the preparation of the inner cores four different materials were selected, between those commonly used in direct compression, according to their specifically compaction behaviour as reported by Roopwani and Buckner [2]. Microcristalline cellulose (MCC) and hypromellose (HPMC) were chosen as model of soft, plastic materials, α Lactose monohydrate (LAC) as model of moderately hard, brittle material and dicalcium phosphate dehydrate (PDC) as model of hard, brittle material. Inner cores composed of the selected materials were prepared at different porosity and analysed in term of mechanical resistance and rheological behaviour (Dynamic Mechanical Analysis). All the inner cores were used to prepared press-coated tablets at different compaction pressure using HPMC as model compound for the outer layer. It has been evaluated the effect of materials tabletability, mechanism of compaction, porosity of the cores, together with the compaction pressure of the final compression, on the mechanical properties of press-coated tablets.
It has been shown that the mechanical properties of the coated tablets are related to the mechanism of compaction, the rheological characteristics and to the porosity of the inner core. Specifically, final tablet with the best mechanical proprieties were obtained operating at high pressure, using high porosity inner cores prepared with high tablettability and ductile materials.

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DEVELOPMENT, CHARACTERIZATION AND IN VITRO SKIN PERMEATION STUDIES OF 8-MOP LOADED SLN

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Psoriasis is considered as a chronic autoimmune inflammatory disorder characterized by thickened inflamed skin lesions covered with scales. Several treatment options are available to alleviate the symptoms of psoriasis by targeting against keratinocytes, inflammation and angiogenesis that lead to expansion of therapeutic strategies in treating psoriasis [1]. Psoralen in combination with ultraviolet A radiation (PUVA) is an FDA recommended therapy for clinical application in the management of severe recalcitrant psoriasis. When activated by UVA light, PSR undergo cycloaddition with pyrimidine bases of nucleic acids forming stable cycloadducts and this mechanism was found to be very effective in treating psoriasis [2;4]. Unfortunately, the presently available topical 8-MOP formulations, such as emulsions, creams and solutions, do not achieve good skin permeability as well as penetration of 8-MOP to deeper skin layers. Delivering higher concentration of 8- MOP to the skin enables the reduction of UVA radiation dose and side effects. Thus, it is required to develop novel drug delivery systems for 8-MOP in order to enhance its skin permeability and consequently increase its safety and efficacy for the treatment of skin disorders [3].

The aim of this study was to develop solid lipid nanoparticles with increased skin permeation and controlled release properties for psoralens. In this study, Compritol 888 ATO or Precirol ATO 5 were chosen as solid lipids. In order to enhance 8-MOP loaded SLN penetration through the skin, a variety of different penetration enhancers, such as Labrasol, Oramix and Transcutol, have been used. SLN were prepared by a hot homogenization technique followed by ultrasonication and were characterized with respect to size, polidispersity index and zeta potential. SLN stability was evaluated on storage at 25ºC for 90 days. The effects of the SLN incorporation on the 8-MOP diffusion through and into the skin were investigated in vitro using vertical Franz diffusion cells and newborn pig skin. The amount of 8-MOP accumulated in stratum corneum, epidermis, dermis and receptor compartment was detected by HPLC using a fluorescence detector.

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In vivo glycemic profile: insulin dry powder inhaler in comparison to Afrezza®

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A pure insulin pulmonary powder (Ins_SD) was developed and patented by the Food and Drug Department of the University of Parma [1]. The aim of this study was to investigate the chemical stability and respirability of this Ins_SD powder loaded in capsules packed in blister. The *in vitro* respirability of Ins_SD was compared to the one of the commercial product Afrezza®. Finally, the glycemic plasma profile of rats was measured after pulmonary insufflation of Ins_SD and Afrezza® at a dose of 10 IU/Kg.

A human recombinant insulin powder for inhalation (Ins_SD) was prepared by spray drying using a mini Spray Dryer Buchi B-290 (Buchi®, CH). The powder was characterized by SEM analysis (FESEM-FIB, Zeiss Auriga Compact,DE) and particle size distribution (Spraytec®, Malvern Instruments Ltd, UK). HPMC capsules size 3 (Quali-V®-I, Qualicaps Europe, ES) were semi-automatically filled with 2 mg of INS_SD powder. The *in vitro* respirability of Ins_SD was assessed using the Next Generation Impactor (NGI) (Copley Scientific, UK) and RS01® high resistance inhaler (Plastiape, IT) at 65L/min to aerosolize the formulation. The *in vivo* study was conducted in rats and the glycemic plasma profile was determined after pulmonary insufflation of Ins_SD and Afrezza® powder. Ins_SD and Afrezza® were blended in Turbula mixer (25 rpm for 5 min) with mannitol spray-dried to have 80 μg of peptide in 2 mg of powder. For SC administration insulin was dissolved in 0.01N HCl/saline 1:9 (solution pH= 4.7). Rats were fasted for 3 h prior to basal glycemic determination then they received a 1g/Kg glucose injection (time zero) and 5 min later 10 IU/Kg of insulin were administered intratracheally (4 per group) using a dry powder inhaler device DP-4 insufflator™ (Penn-Century, Inc, Philadelphia, US).

The results from this study show that Ins_SD presented higher respirability and favourable stability behaviour than Afrezza® [2]. Ins_SD powder delivered dose was >95% and the FPF lower than 5 μm 91%. The MMAD value was 0.85 μm. Afrezza® powder was successfully emitted from the device (more than 95%) and MMAD value was 3.2 μm. The Ins_SD powder, filled in Quali-V®-I capsules can provide long term stability at room temperature maintaining the good aerodynamic performance. It was demonstrated that Ins_SD provided a similar profile of glucose control as Afrezza® ($p>0.05$) and were capable to more efficiently decrease the glucose level at min 15 similar to the one of subcutaneous administration.

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GLYCOGEN-BASED CATIONIC NANOVECTORS FOR siRNA DELIVERY

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Polglumyt is a highly purified form of glycogen, characterized by high water solubility (30% w/v), which is accompanied by very moderate increase in viscosity due to the hyperbranched nature of the macromolecule. Its dendrimeric structure, appropriately functionalized, makes it an alternative to current synthetic gene delivery agents such as dendrimers or hyperbranched polymers.[1] Polglumyt Policationic Derivates (PPDs) are hyperbranched nanocarriers for nucleic acids, which also combine natural origin, degradability, negligible toxicity and good loading capacity. PPDs are synthesized starting from a purified form of glycogen by introducing, into polymer chains, cationic or positively ionizable groups, which can electrostatically interact with nucleic acids. The present work describes the preparation of PPDs, and their characterization as carriers for nucleic acids. PPDs were prepared by reaction of Polglumyt with different N,N-dialkylaminoalkyl halides under alkaline aqueous conditions. PPDs were synthesized in different N,N-dialkylaminoalkyl halides/Polglumyt molar ratios (0.043; 0.25; 0.50; 1.00; 2.00). All derivates were characterized by nuclear magnetic resonance, gel permeation chromatography and shear rheometry. Dynamic Light Scattering and Laser Doppler Velocimetry were performed with the Zeta Nanosizer ZS90 instrument. DLS was used to measure hydrodynamic size, size distribution of PPD/nucleic acid complexes and to verify the presence of aggregation phenomena. Measurements for ζ potential were performed by LDV technique using PPDs solutions in pH 4.5 Phosphate Buffer. The PPDs were also characterized regarding their pK_a values, using a Mettler Seven Excellence Multiparameter, titrating PPDs aqueous solution with a 1N NaOH solution. PPDs were synthetized in good to excellent yields (60-80%). All the derivates are characterized by the presence of two different basic groups: the first one has a pK_a above the physiological pH, which should guaranty the binding with nucleic acids, whereas the second one should promote endosomal escapes by proton sponge effect. Optimizing Polglumyt derivates structure in terms of extent of derivatization and nature of the cationic groups, we could achieve high RNA loadings with negligible aggregation, and good protection from nuclease degradation with negligible cytotoxicity.

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COENZYME Q_{10} NANOSUSPENSIONS FOR PULMONARY ANTIOXIDANT THERAPY

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Coenzyme Q_{10} (CoQ_{10}) is a fat-soluble vitamin K-like antioxidant. Due to its poor water solubility, it has been suggested that CoQ_{10} bioavailability might be improved by incorporating the substance into submicron particles [1]. It is currently marked in the US as dietary supplement, and widely used in the treatment of cardiovascular disorders [2]. As antioxidant, CoQ_{10} has also been studied as adjuvant treatment for cancer to prevent cancer and reduce adverse effects of chemotherapy [3].

The aim of this work was the production by high-pressure homogenization, characterization and stability study of three CoQ_{10} nanosuspensions designed to be delivered to the lungs by nebulization.

Three surfactants, i.e. lecithin, PEG32 stearate and Vitamin-E TPGS, were selected to stabilize CoQ_{10} formulations. Preparations were identified as nanosuspensions (hydrodynamic diameter in the range 35-60 nm and ζ potential between -20 and -25 mV): the smallest particles were obtained with Vitamin-E TPGS and denoted a lipid core/surfactants-shell structure by Small Angle X-ray scattering analysis. The CoQ_{10} delivered from an air-jet nebulizer was in all the cases around 30% of the loaded dose. The nanosuspension containing PEG32 stearate presented the highest Respirable Fraction (70.6% ± 5.1) and smallest Median Mass Aerodynamic Diameter (3.02 µm ± 0.49). Stability tests showed that the most stable formulation, after 90 days at room temperature and at 5°C, was the one containing Vitamin-E TPGS, followed by the CoQ_{10}-lecithin formulation. Interestingly, those formulations were demonstrated to be suitable also upon nebulization with other type of devices, such as ultrasound and vibrating mesh nebulizers.

Studies focused on *in vitro* cellular toxicity of the formulations and their single components using A549 human lung cells showed non-obvious cytotoxicity for the formulations containing lecithin and PEG32 Stearate. Vitamin-E TPGS, already in use for several drug delivery applications (i.e. ocular), when alone proved to be able to damage the plasma membrane, nevertheless, cell damage was decreased when Vitamin-E TPGS was present in the formulation with CoQ_{10}.

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HyDrO-DiAb: Polyphenols loaded in Hydrogel in the treatment of diabetic foot ulcer

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INTRODUCTION

Diabetes affects approximately 170 million people worldwide, including 3 million 200 thousand people in Italy [1] and, its effects, include long-term damage such as blindness, nephropathy and/or neuropathy with risk of foot ulcers and amputation [2].

The purpose of this research is to develop and to characterize a biodegradable polymeric Hydrogel, which is able to absorb exudate and to exert an anti-oxidant activity and to stimulate tissue regeneration process.

KEYWORDS: Diabetes – Foot ulcer – Hydrogel – Natural substances – Delivery

EXPERIMENTAL PART

In this work, Chitosan, Carboxymethyl cellulose, $AgNO_3$ and Ascorbic Acid, in a phosphate buffer solution (pH 7.4), were dissolved. At the end of this step, Olive Leaf dry extract and Camellia Sinensis leaf extract were added to the mix of dissolved polymers. To obtain the hydrogel (HyDrO-DiAb), the obtained mixture was dried at 40 °C into sterile glass Petri dishes.

Several tests were carried out in the aim to investigate the physical and biological properties of HyDrO-DiAb including: evaluation of swelling behavior and water retention; MTT assay for cell viability; Antioxidant properties and *in vitro* Wound Healing assay.

RESULTS AND DISCUSSION

HyDrO-DiAb shows fast rate of absorption in the first 80 min followed by a slow increase in the next 40 min (Figure 1) and shows decrease in moisture content (R_h) with time (Figure 2), after 16 h the total loss was approximately 70-80%. Then, MTT Assay in 3T3 fibroblast cell line was performed and the obtained results (Figure 3) demonstrate that HyDrO-DiAb is not cytotoxic. HyDrO-DiAb shows 47.3 ± 0.1% (Figure 4) and 37.4 ± 0.4% (Figure 5) DPPH and ABTS inhibition ability, respectively. In the last test, HyDrO-DiAb confirms its healing property (Figure 6) and, in particular, its ability to below *in vitro* wound closure. The wound closure percentage with 100 μg/mL and 200 μg/mL of HyDrO-DiAb was 67 ±0.3% and 80.2 ± 0.5% respectively. In contrast, cells treated with Chi-CMC showed 21.4 ± 0.7% wound closure.

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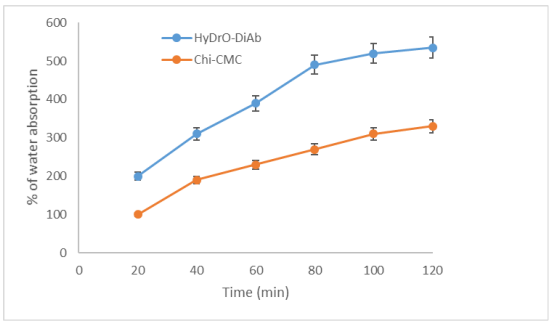

Figure 1: Water absorption capacity (%)

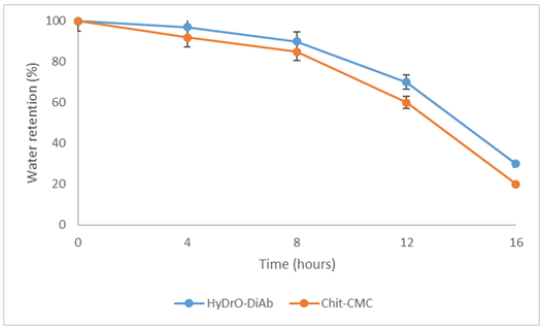

Figure 2: Water retention capacity (%)

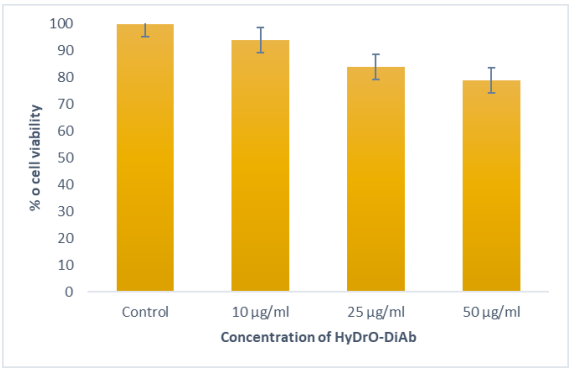

Figure 3: MTT test on 3T3 fibroblast cell line with different concentrations of HyDrO-DiAb.

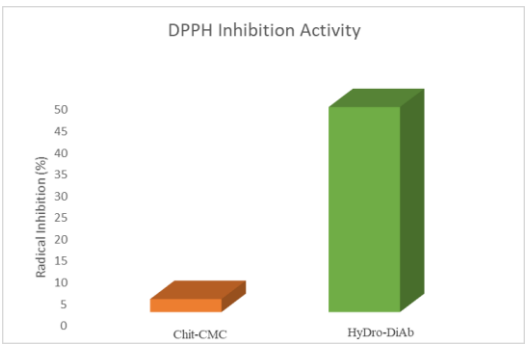

Figure 4: DPPH inhibition activity evaluation

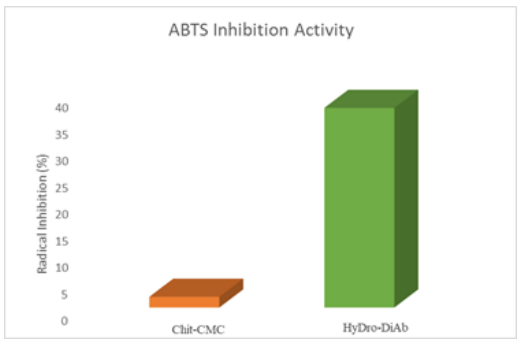

Figure 5: ABTS inhibition activity evaluation

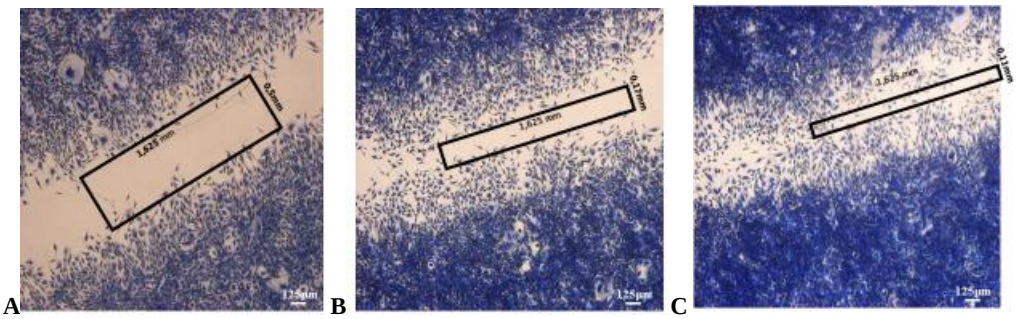

Figure 6: Cells were treated with Chi-CMC (a), HyDrO-DiAb (100 µg/ml) and with HyDrO-DiAb (200 µg/ml).

Freeze drying optimization of lipid nanoparticles for naproxen delivery: formulation, characterization and effect of cryoprotectant agents.

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Solid lipid nanoparticles (SLNs) represent an alternative colloidal carrier system to polymeric nanoparticles, liposomes and emulsions [1]. A crucial point for the use of the SLNs as colloidal drug carriers is their physical and chemical long-term stability [2,3]. For such cases, it is highly desirable to have a freeze-dried SLN formulation available [4,5]. The aim of the project, therefore, was to formulate SLN obtained by the elimination of dilution water during the cooling process, and to study their characteristics after undergoing the lyophilization process. Therefore, the lyophilization process was investigated and optimized and the effect of drug incorporation was evaluated too. SLNs were prepared using the solvent-diffusion technique and then were freeze-dried. Softisan 100 and poloxamer 188 were used as lipid matrix and surfactant, respectively. Freeze-thaw cycles were carried out, as a pre-test, to study the protective effect of various types and concentrations of cryoprotectants (e.g. glucose,mannitol and trehalose with the concentration from 0.5% to 4.5%). Glucose proved to be most effective in preventing particle growth during freezing and thawing and also in the freeze-drying process. Changes in particle size distribution during lyophilization could be minimised by optimising the parameters of the process; the optimum concentration of glucose was 4%. Naproxen was incorporated in these SLN formulations, because it guaranteed the best redispersion performance after lyophilization.

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HYBRID SELF-ASSEMBLING NANOPARTICLES FOR THE DELIVERY OF microRNA

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MicroRNA (miRNA) are non-coding RNAs involved in regulation of gene expression and potential therapeutic agents in the treatment of diseases. In different forms of cancer, e.g. glioblastoma (GBM), miRNAs can control the occurrence of resistance against chemotherapeutics. However, the therapeutic use of miRNA is hampered by biopharmaceutical issues, requiring the use of *ad hoc* developed delivery systems [1]. Previously, a novel formulation consisting on hybrid self-assembling nanoparticles (SANPs) for the delivery of anionic drugs, e.g. bisphosphonates, in the treatment of glioblastoma (GBM), have been developed [2]. The aim of this work was development of SANPs for the delivery of miRNA, e.g. miR603, in the treatment of GBM.

The SANPs were prepared by mixing miRNA and Calcium/Phosphate dispersion (CaP NPs), followed by mixing with PEGylated liposomes (PL). In order to optimize the formulation, different lipid compositions were tested. In particular, liposomes composed of different cationic lipids, with or without neutral lipids, and with two different PEGylated lipids were prepared. All formulations were characterized in terms of mean diameter, polydispersity index, zeta potential, miRNA encapsulation, NPs stability in BSA, serum and red blood cells, NPs hemolytic activity. Moreover, the SANPs were tested *in vitro* (cytotoxicity, uptake, Real Time PCR analysis) on U87MG and LN229.

The majority of the formulations showed a mean diameter lower 170 nm and a polydispersity index lower than 0.2. The zeta potential was positive (between +10mV and +45mV) and the miRNA encapsulation efficiency was between 75% and 100%. The formulations were stable in BSA and serum four hours. Finally, the majority of the formulations showed a hemolysis lower than 2%. *In vitro* studies on two different cell lines, allowed to select the formulations that were no toxic, which were tested in the following miRNA intracellular uptake studies. SANPs based on cationic lipid 1,2-dioleoil-3-trimetilammonio-propano (DOTAP) combined with PEGylated lipid N-palmitoyl-sfiingosina-1-{succinil [metossi (polietilen glicole) 2000]} (PEG-Cer_{16}) lead to the highest miR603 delivery in GBM cells and was considered suitable for the following in vivo studies.

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DESIGN OF DRUG DELIVERY SYSTEMS FOR THE NOSE-TO-BRAIN ADMINISTRATION OF GERANIOL

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The aim of this study was the design of drug delivery systems for the nose-to-brain administration of Geraniol (Ger), a monoterpene present in various essential oils, which exhibits strong anti-inflammatory and anti-oxidant effects [1]. Ger has been proposed for the treatment of neurodegenerative disorder, such as Parkinson's disease, associated with both inflammatory response and release of reactive oxygen species [2]. However, its activity is impaired by the fast metabolism following the oral administration. Preliminary studies demonstrated that Ger reaches in large amounts the brain after intranasal administration, but causing a huge damage to the nasal mucosa, destroying it. Therefore, a protecting delivery system is required to encapsulate Ger and to safeguard the epithelium.
Different formulations were designed starting from several materials such as lipids (Compritol, Gelucire, Isopropyl myristate, triacetin), polymers (PLGA and Gelatine) and cyclodextrins (CDs), obtaining Ger-loaded nanoparticles (Ger-NPs) or Ger-CDs complexes. All the carriers were freeze-dried and characterized before and after the freeze drying process regarding the size by Photon correlation spectroscopy (PCS) and the drug loading by HPLC analysis.
The results demonstrated that all the fresh prepared Ger-NPs were small and homogenous in the size and exhibited a high drug loading. In order to obtain more *in vitro* stable formulations, Ger-NPs were freeze-dried but, after this process, while no modification in the size was observed, the drug loading became negligible, probably owing to the volatility of the drug. On the contrary, in the case of the inclusion complexes composed by β-CD/HP- β-CD, the encapsulation efficiency remained high (about 80%). Therefore, these formulations were characterized by electron microscopy, *in vitro* drug release, infrared (IR) spectroscopy, CHN analysis and differential scanning calorimeter (DSC). The characterization analyses showed that both the inclusion complexes were successfully formed and that their *in vitro* stability was suitable for a nose-to –brain administration.
Further studies are needed to evaluate the potentialities of these carriers for an *in vivo* intranasal administration in Parkinson's disease models.

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