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7th AItUN

Annual Meeting

March 8-9, 2013

Perugia, Italy

NEW FRONTIERS IN LIVING CELL ENCAPSULATION

Abstract Book

New Frontiers in living cell encapsulation

March 8-9, 2013

Perugia, Italy

Book of Abstracts

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Final Program

Friday, March 8

9.30-11.30 **Registration**

Opening Session

11.30 **Welcome and Opening Remarks**

Prof. Maurizio Ricci, A.It.U.N. Faculty Advisor

Prof. Benedetto Natalini, PhD Program Coordinator of Chemistry and Technology of Drug PhD and Director of the Department of Chemistry and Technology of Drug

Prof. Carlo Rossi, President of Consorzio TEFARCO Innova

Prof. Franco Alhaique, President of ADRITELF

Emanuele Cassetti, A.It.U.N. President

Session 1

Chair: Prof. Maurizio Ricci, Emanuele Cassetti

11.45 **Plenary Lecture** - Prof. Riccardo Calafiore (University of Perugia) Alginate-based microcapsules for pancreatic beta- cell substitution therapy in T1DM: from bench to bedside.

12.30 **Invited Lecture** - Ing. Fabiola Munarin (Politecnico di Milano) Pectin microspheres as cell carriers for bone tissue regeneration.

13.00-15.30 **Lunch and Poster Session**

Session 2

Chair: Barbara Colzani, Mariateresa Stigliani

15.30 **Invited Lecture** - Prof. Thomas Czerny (University of Applied Sciences of Vienna) Magnetic field -controlled gene expression in encapsulated cells.

16.00 **Invited Lecture** - Prof. Gianluigi Mauriello (University of Naples "Federico II") Microencapsulation for preservation of bioactive components in foods.

16.30 **Technical Presentation** - Dr. Micheal Whelehan (Büchi Switzerland) B-395 Vibration Technology applied to cell encapsulation.

Saturday, March 9

09.00 **Breakfast**

Session 4

Chair: Barbara Albertini, Virginia Campani, Ilaria Franceschini

10.00 **Oral Presentation:** In vivo toxicity and teratogenicity evaluation of plant virus nanoparticles - Agnese Blandino (Università degli Studi di Perugia).

10.20 **Oral Presentation:** Hyaluronic acid based film loaded with cisplatin for malignant pleural mesothelioma: a novel loco - regional therapy – Luca Castrati (Università degli Studi di Parma)

10.40 **Oral Presentation:** Role of the mobile phase composition in the chromatographic enantioseparation of pharmaceutically relevant compounds – Federica Ianni (Università degli Studi di Perugia)

11.00 **Oral Presentation:** Application of vibrating technology for microencapsulation of bacterial cells and bacteriocins – Annachiara De Prisco (Università degli Studi di Napoli).

11.20 **Oral Presentation:** Resveratrol-loaded nanocarrier for inner ear delivery – Musazzi Umberto Maria (Università degli Studi di Napoli).

12.30 **Concluding Remarks and A.It.U.N. Member Assembly**

Invited Lectures

Prof. Riccardo Calafiore

Biography

1979: Degree in Medicine and Surgery (M.D.) with full marks and "maxima cum laude", University of Perugia, School of Medicine. 1984: Specialization in Internal Medicine, with full marks and "maxima cum laude", Post-graduate Internal Medicine Specialty School, the University of Perugia. 1987-89: Adjunct Professor of Endocrinology, Endocrinology and Metabolism, Post-graduate Specialization School, the University of Perugia. 2002-03: Professor of Endocrinology, Post-graduate School in Endocrinology and Metabolic Diseases, University of Perugia School of Medicine.

Dr. Calafiore is the author of 200 full papers (plus over 120 communications), 90 of which are PubMed impacted, mainly focused on pancreatic islet transplantation for the potential cure of insulin-dependent diabetes mellitus, most of which reported in peer-reviewed Journals.

Dr. Calafiore is one of the world leaders in the scientific and academic community engaged in islet as well as other insulin producing cells, as well as other endocrine cell types transplantation for the cure of type 1 diabetes mellitus. In particular, he has developed pioneering work on artificial membranes (microcapsules) for immunoprotection of the cell grafts with no general, pharmacologic recipient's immunosuppression. In recent years he has created an Interdisciplinary Laboratory for Endocrine Cell transplant and Bioartificial Organs that actually is engaged in the following frontier research studies: 1) Induction of acquired recipient's immunotolerization, by graft of neonatal porcine Sertoli cells within microcapsules, in several experimental animal models of autoimmune/inflammatory-based disorders : a) type 1 diabetes mellitus; b) autoimmune colitis; c) autoimmune encephalomyelitis; muscular dystrophy; d) Laron syndrome; e) type 2 diabetes mellitus; f) hematologic proliferative disorders (leukemia). 2) Use of adult mesenchymal stem cells originally isolated from the post-partum, umbilical cord Wharton Jelly, as an immunomodulatory and substitutive cell model for tissue engineering in type 1 diabetes and other applications. 3) Development of a new generation of immunomodulatory minimal size, alginate-based microcapsules for immunoisolation of several cell grafts and macroscopic scaffold for tissue regeneration.

ALGINATE-BASED MICROCAPSULES FOR PANCREATIC ISLET BETA-CELL SUBSTITUTION THERAPY IN TYPE 1 DIABETES MELLITUS : FROM BENCH TO BEDSIDE

Riccardo Calafiore

Department of Internal Medicine, Division of Endocrinology and Metabolism, University of Perugia, Perugia, Italy

Type 1 diabetes mellitus is an autoimmune disease that on the besis of a genetical background, results in selective killing of insulin producing Beta-cells : the ensuing hyperglycemia will coincide with the clinical onset of the disease. While exogenous insulin supplementation allows these patients to survive, substitution of the diseased/destroyed Beta-cells would be the only approach to cure this metabolic disorder. Alginate-based microcapsules were initially proposed since the '80s as immunoprotective shields to circumvent pancreatic islet graft-directed immune destruction in rodents with artificially-induced diabetes mellitus. These spherically shaped microbeads measuring an average 600-800 microns in equatorial diameter consisted of a gel core, containing the cells, and an outer coat comprising a polyaminoacidic layer and external final alginate layer to provide these beads both immunoselectiveness and biocompatibility. Throughout the years, we have developed in our laboratory several, original strategies to devise new microencapsulation technologies to fabricate increasingly performing membranes. In particular we aimed to : 1) generate methods for ultra-purification of the basic raw alginic powder, in order to achieve a "clinical grade" product, according to the U.S. FDA guidelines, to be employed in humans; 2) improve and perfect size microcapsules size in order to better distribute them, upon graft, in the recipients' peritoneal cavity or other graft sites; 3) explore other insulin producing cell phenotypes, besides islets, whose availability, with special regard to human is quite restricted, to enable use of more affordable and virtually inexhaustible cell sourcing. All these items have been successfully accomplished throughout the recent past years. In particular we have been granted permission from the Italian Ministry of Health to initiate a phase I, pilot clinical trial of microencapsulated human islets into nonimmunosuppressed patients with long standing, strictly insulin-dependent type 1 diabetes mellitus. 4 of these cases have been completed so far and monitored for 5 years after transplant. The graft procedure consisted of a simple intraperitoneal injection with delivery of the capsules suspension, under local anesthesia. Graft function was obtained in all recipients, as documented by appearance and sustenance of plasma C-peptide levels that were absent, and decline through 75% of the daily exogenous insulin dose supplementation. While only one patient suspended insulin administration transiently, in all HbA1c levels

significantly declined and acute episodes of hypoglycemia disappeared. Upon 5 years of follow-up, when all patients had returned to their intial daily insulin schedule, a biopsy in patient #1 showed the presence of intact capsules that contained mostly necrotic debris although some live cells could be detected. In parallel, and on a pre-clinical level, we have successfully used other cell types such as Sertoli cells extracted from pre-pubertal pig testes, that unpon microencapsulation and graft have been able to cure near to 80% of NOD mice with spontaneous autoimmune diabetes similar to human. This cells changed the immune system of these animal shifting from the auto-aggressive mode to acquired immune tolerance, thereby allowing regeneration of new Beta cells, preceded by activation of key genes like NGN3 and PDX-1. We are recently upgrading these observations to primates and two of them are being transplanted. Finally we have developed a method for retrieval of mesenchymal adult stem cells from the post-partum human umbilical cord Wharton Jelly. These cells may be conditioned, in vitro, to evolve towards several cell phenotypes, and preliminarily we have gathered evidence that they may be associated with islet-like structures able to produce insulin. Studies in diabetic mice with encapsulated mesenchymal cell grafts are actually in progress.

Ing. Fabiola Munarin

Biography

Fabiola Munarin was born in Milan in 1983. She received her Master degree in Biomedical Engineering from the Politecnico di Milano in 2007 with her thesis "Preparation and characterization of natural polymer-based microcapsules for autologous cell delivery". In 2011 she received the European Ph.D. (cum laude) in Bioengineering.

At present she is a Post-doctoral fellow in Bioengineering at Politecnico di Milano, and she works in BioMatLab Laboratory. Her research topic is on the use of natural polymers as carriers for drug, cells or gene delivery. In particular, she is focusing on the preparation of micro-structured hydrogels for autologous cell immobilization.

She is author of several international peer-reviewed papers and of an international patent.

PECTIN MICROSPHERES AS CELL CARRIERS FOR BONE TISSUE REGENERATION

Pectin is an anionic polysaccharide extracted from the plant cell walls and mainly used as a food thickener or gelling agent. The carboxyl groups of pectin backbone are involved in the gelling process, with the formation of the “egg box” structure in the presence of divalent cations.
Pectin microspheres were obtained either with a coaxial air flow system and with an electrostatic bead generator, and cell immobilization was performed using line cells (C2C12 and MC3T3-E1).
Pectin was further modified to address for bone regeneration: oxidation was performed to increase its degradability, and the RGD sequence was grafted to improve cell adhesion. Treatments in SBF were made to create a biomimetic environment, simulating the natural inorganic phase of the bones.
Preosteoblasts (MC3T3) and human mesenchymal stem cells (hMSCs) were immobilized in RGD-coupled pectin microspheres, and the in vitro and in vivo characterization demonstrated that cells were sprout inside the microspheres and organized in 3-dimensional structures, regenerating the natural extracellular matrix and showing osteogenic behaviour.
On the overall, the obtained results indicate that pectin microspheres can be considered attractive cell carriers for bone regeneration.

Prof. Thomas Czerny

Biography

After the study of pharmacy and a PhD in biophysical chemistry Prof. Czerny worked as an assistant professor at the University of Agricultural Sciences in Vienna. He then moved as a postdoc to the Institute of Molecular Pathology in Vienna (group of Meinrad Busslinger) where he spent 6 years. After a short stay at the Hubrecht Laboratory at Utrecht he started his own group at the University of Veterinary Medicine in Vienna (Institute of Animal Breeding and Genetics). Research topics are cellular signalling pathways with special emphasis on the Wnt and the heat shock pathway. Since 2010 he keeps an endowed professorship for Functional Genomics at the University of Applied Sciences, FH-Campus Wien.

MAGNETIC FIELD -CONTROLLED GENE EXPRESSION IN ENCAPSULATED CELLS

T. Czerny

University of Applied Sciences, FH Campus Wien, Department for Applied Life Sciences, Vienna, Austria

Encapsulation of cells allows the production of therapeutic substances at high concentrations exactly where they are needed in the patient. This restriction to a certain area reduces drug-specific side effects in the remaining body. But as for most drugs, the dose critically determines the effect. Regulation from the outside is therefore necessary to adjust expression to the required therapeutic window.

Several inducible expression systems have been established, which mainly rely on small molecules as inducers, such as hormones or antibiotics. The application of these inducers is difficult to control and the effects on gene regulation are slow. We developed a novel system for induction of gene expression in encapsulated cells. Based on a heat inducible promoter the potential therapeutic genes are expressed in a heat dependent manner. During encapsulation the cells are mixed with magnetic nanoparticles. The nanoparticles produce heat when subjected to an alternating magnetic field. This results in elevated temperatures in the capsules, which then induce gene expression. The system allows high expression levels and can be regulated in a fine tuned manner and within short time.

Prof. Gianluigi Mauriello

Biography

Gianluigi Mauriello is Associate Professor of Food Microbiolgy at the Department of Agricultural Science, University of Naples Federico II. Graduate in Agricultural Science.
He is author of more of fifty peer-reviewed papers.
Following the relevant research activities:
• metabolomic approach to evaluate differences among strains within a lactic acid bacteria population;
• metabolomic approach to evaluate the technological performances and to differentiate on the base of geographical origin a set of natural whey cultures used for the production of water buffalo mozzarella cheese;
• use of bacteriocins, essential oils and bacteriophages to control pathogenic and spoilage bacteria in food by using them as additives or by functionalization of food packaging materials;
• proteomic approach to detect up- and down-regulation of stress response genes and related proteins in order to understand the mechanism of action of essential oils in Salmonella enterica ser. Thompson;
• microencapsulation of bacterial cells and other bioactive compounds.

MICROENCAPSULATION FOR PRESERVATION OF BIOACTIVE COMPONENTS IN FOODS.

G. Mauriello, C. Malmo and A. La Storia

Department of Agricultural Science, University of Naples Federico II

Microencapsulation is a technological process applied in different industrial fields. By this process it is possible to coat or entrap liquid or solid substances in a material to obtain a solid product constituted by microparticles. They can have a diameter ranging from 1 to 2000 μm and can classified as microcapsules or microspheres according to their inside structure. Microcapsules are represented by a core of functional/active compound surrounded by an external shell of polymeric or lipid material. Microspheres are matrices systems in which the functional/active compound is homogeneously dispersed into a matrix of polymeric or lipid material (de Vos et al., 2010, Int. Dairy J. 20: 292-302.). Probiotic bacteria are optimal subjects for microencapsulation, an effective system for their protection both from unfavourable conditions of gastrointestinal (GI) tract and some process conditions in food industry (Rokka and Rantamaki, 2010, Eur. Food Res. Tech. 231: 1-12). In fact, these conditions are frequently the main reason of dramatic reduction of numbers and viability of bacterial populations ingested under different formulation, with the consequent loss of probiotic value. In this work, cells of Lactobacillus reuteri DSM 17938 was microencapsulated by spray drying in alginate beads and than coated with chitosan. Briefly, a broth culture of Lactobacillus reuteri DSM 17938 was centrifuged and the pellet suspended in 1% sodium alginate. This preparation was subjected to a spray drying process by the Mini Spray Dryer (BUCHI, Switzerland) to obtain the so-called "un-cross-linked microcapsules". After a treatment with 1% calcium chloride solution in Ultra-Turrax homogenizer the "cross-linked microcapsules" was obtained. Finally, "cross-linked microcapsules" was collected by centrifugation, stirred in a solution of 2% chitosan in 1% acetic acid e further centrifuged to obtain the so-called "co-cross-linked microcapsules". All microcapsules were morphologically characterized by fluorescence microscopy after viable staining (LIVE/DEAD® BacLight™ Bacterial Viability Kit). Moreover, microencapsulated cells were examined for their surviving after exposure to different thermal stress (60°C for 3 min, 70°C for 2 min, 80°C for 2 min, 80°C for 5 min) and simulated GI conditions (phosphate buffer at pH 2.5 and MRS supplemented with 0.3% bile salts). Finally, microcapsules containing Lactobacillus reuteri DSM 17938 cells was used in the preparation a potential probiotic chocolate soufflé. Results showed a 15.6% of surviving at 60°C for 3 min of "co-cross-linked microcapsules" compared to 2% of surviving at the same condition of free cells. Moreover, cells were significantly protected in the "co-cross-linked

microcapsules" after exposure to GI conditions and the rate of surviving was 10 times higher than free cells after baking of chocolate soufflé.

Dr. Micheal Whelehan

Biography

Micheal Whelehan obtained his bachelor's degree from Dublin City University (DCU) and received his Ph.D form the same institution in 2010, in which his work mainly focused on producing microcapsules (using prilling by vibrating technology) for application in biotechnological and medical processes. During his Ph.D he spent nearly two years as a visiting scholar to the Ecole Polytechnique Fédérale de Lausanne (EPFL) in the laboratory of Prof. Urs von Stockar performing the aforementioned work.

In 2010 Dr. Whelehan began a post-doc position, again in Dublin, where he spent nearly 2.5 years continuing the work he performed during his Ph.D. In addition he was involved in setting up a laboratory dedicated to micro/nano – encapsulation research at an academic and industrial level. In this time he was also involved in numerous collaborations with many companies, with regard to applying Encapsulation technology at an industrial level.

Since October 2012 Dr. Whelehan has being working as a product specialist in Encapsulation and Spray Drying Technology at BÜCHI Labortechnik AG, in Flawil Switzeland. BÜCHI are world leaders in Spray Drying and Encapsulation (using the prilling by vibration technique) technologies for lab-scale R&D work.

Dr. Whelehan's research interests (using Encapsulation and/or Spray Drying technologies) include:

- Cell encapsulation
- Generation of artificial implants for transplantation
- Drug delivery and recovery
- Incorporation of bioactives in (functional) foods
- Bioprocessing (downstream processing and in-situ product recovery)
- Environmental applications (wastewater treatment)
- Development of new encapsulation technologies to overcome presentation limitations, and• Nano-encapsulation.

Student Oral Presentations

IN VIVO TOXICITY AND TERATOGENICITY EVALUATION OF PLANT VIRUS NANOPARTICLES

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Purpose. Plant virus nanoparticles are showing great potential as delivery system for diagnostics and therapeutics [1]. The aim of the study was to evaluate the toxicity and teratogenicity of Potato Virus X (PVX) and Tomato Bushy Stunt Virus (TBSV) by direct yolk sac injection in early chick embryos.

Methods. Five different concentrations of PVX and TBSV nanoparticle (NP) suspensions were injected in the yolk sac 16h after incubation and chick embryos were sacrificed 26h later. Toxicity was evaluated both by comparing dead embryos and somite number with negative controls. Teratogenicity was evaluated by analyzing somites, vascular area, and neural tube deformities. Black carbon NPs and retinoic acid were used as positive controls for toxicity and teratogenicity.

Results. Negative controls (i.e. untreated embryos and vehicle) showed a somite number (14.06 ± 1.12 and 15.6 ± 2.7) compatible with that reported by Hamburger and Hamilton at the same stage [2] and dead embryos were lower than 5%. At concentrations ranging from 1 ng/embryo to 10 µg/embryo, the 2 toxicity indicators revealed no significant differences ($p>0.05$) between PVX and TBSV treated embryos and the negative control. The same concentrations did not grant signs of teratogenicity. On the contrary, positive controls resulted appropriate since 50% and 25% of deaths as well as 100% and 51% of malformations were recorded for black carbon NPs and retinoic acid, respectively.

Conclusions. Even though additional results and tests in other animal models are mandatory to have conclusive opinion on the safety of these NPs, the reported results evidenced their safety in chick embryo model.

[1] Grasso S., Santi L., Viral Nanoparticles as macromolecular devices for new therapeutic and pharmaceutical approaches, Int. J. Physiol. Pathophysiol. Pharmacol., 2:161-178 (2010).
[2] Hamburger V., Hamilton H.L., A series of normal stages in the development of the chick embryo, J. Morphol., 88:195:49-92 (1951).

HYALURONIC ACID BASED FILM LOADED WITH CISPLATIN FOR MALIGNANT PLEURAL MESOTHELIOMA: A NOVEL LOCO - REGIONAL THERAPY.

[1]L. Castrati, [2]S. Barbieri, [3]F. Sonvico, [1]P. Colombo

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[3] Graduate school of health, Sydney

Purpose. To produce and characterize a hyaluronic acid-based film loaded with cisplatin for a loco-regional therapy of malignant pleural mesothelioma.

Methods. Polymeric films were produced for the local delivery of anticancer drugs. Briefly after preparing the filmogenic solution, the hyaluronic acid was added. Lastly the solution was loaded with cisplatin at a concentration of 0.3% w/v. Drug release profile was determined both in vitro and in vivo. For in vitro tests Franz cells were used, with a buffer solution as release medium. For in vivo experiments, female Sardinian sheep were chosen as testing subject. After general anesthesia a lateral thoracotomy and a left pneumonectomy ware performed. Thereafter, the treatment was randomly administered: intravenous cisplatin, intrapleural cisplatin, hyaluronate-cisplatin. Controls (pneumonectomy alone) were used for comparison. Blood samples were taken as scheduled. The animals were euthanatized after 216 h: serum and tissue samples were considered for analysis. Primary endpoint was plasmatic cisplatin concentration measured by IPC-mass spectrometry. Secondary endpoints were tissue drug concentration and treatment-related toxicity. Data are given as mean.

Results. In vitro drug release test showed an interesting profile, since 50% of the cisplatin was released in 24h and 100% in 60 h. The in vivo results, based on a total of 21 animal treated with 3mg/kg of cisplatin, show that after 30' from the treatment the plasmatic concentration of the animals with hyaluronic acid - cisplatin was 192 ng/ml, which was lower than the intrapleural cisplatin application (905 ng/ml) and intravenous cisplatin administration (3244 ng/ml). At 216 h, plasmatic cisplatin concentration after intrapleural hyaluronate-cisplatin treatment was 2326 ng/ml, while it was 1133 ng/ml in the intrapleural treatment and 913 ng/ml in the intravenous administration. This data show that the hyaluronic acid based film can delay the release of the drug, with low concentrations of cisplatin immediately after the treatment, and high and steady concentrations at longer timescales. No hematological toxicity was registered, while the toxicology profile of this pharmaceutical form shows that there is an improvement in comparison with the other cisplatin therapies.

Conclusions. Intrapleural polymeric films containing cisplatin assured higher pleural drug concentration than cisplatin solution without increasing systemictoxicity after 10 days, proving that this formulation is suitable for clinical therapy.

APPLICATION OF VIBRATING TECHNOLOGY FOR MICROENCAPSULATION OF BACTERIAL CELLS AND BACTERIOCINS

A. De Prisco, D. Maresca, G. Mauriello

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Purpose. Microencapsulation is a promising technology useful for preserving bacterial cells and (bio)active compounds from surrounding conditions. Different main technologies have been described: coacervation, emulsification, spray drying, spray cooling and extrusion (De Vos et al. 2010, Int. Dairy J. 20:292-302). Recently, a mechanical procedure named vibrating technology was described. The aim of this study was microencapsulation of Lactobacillus reuteri DSM 17938 cells and nisin in Ca-alginate by vibrating technology.

Methods. A solution containing the product to be encapsulated and encapsulating polymer matrix, i.e. 2% alginate, is forced into a pulsation chamber and then extruded flowing through a nozzle. The droplets are undergone to an electrical field generated between the nozzle and the electrode in order to charge their surface. Electrostatic forces led to the droplets repulsion. Dropping of the beads in calcium chloride led to microcapsules formation by ionotropic gelation. Beads size depends mainly by nozzle diameter but also by superimposed vibration frequency, amplitude flow rate and physical properties of polymer-product solution.

Results. Lactobacillus reuteri DSM 17938 and nisin were encapsulated in Ca-alginate matrix. Light microscope immages show homogenous and spherical shaped capsules with a diameter of 150 µm. L. reuteri and nisin microcapsules were stained with Bac-light and Fluorescein isothiocyanate (fitc) respectively. Fluorescence microscope images display a high ratio of cell viability related to L. reuteri microcapsules and a high encapsulation efficiency for both microcapsules. Test in vitro shows that microencapsulation process preserve viability of L. reuteri cells and nisin bioactivity under different stress conditions.

Conclusions. Vibrating technology could be a valid technique for the production of different types of alginate-based microcapsules for application in biotechnological processes. This methodology has capability to produce small (<200 µm), mono-dispersed, homogenous and spherical capsules, with a narrow size distribution, using a short production time, under mild and simple conditions, low costs and high encapsulation efficiency (Whelehan and Marison 2011, J. Microencapsul. 28:669-688). Furthermore, this technique can be adapted to microencapsulation of several (bio)active compounds because of absence of high temperature application.

ROLE OF THE MOBILE PHASE COMPOSITION IN THE CHROMATOGRAPHIC ENANTIOSEPARATION OF PHARMACEUTICALLY RELEVANT COMPOUNDS

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Purpose. The recognition of chirality as a new asset in drug development has an enormous effect on the product pipelines of the major players in the pharmaceutical industry. Accordingly, enantioselective analytical assays play a key role in the development of chiral drug candidates throughout the entire development cycle, including the quality control of the formulated drugs. Suitable analytical procedures are indeed required to demonstrate that the manufacturing process doesn't induce unacceptable changes in enantiomer purity of the final products, and that stereochemical integrity may be maintained for the proposed shelf-lives.

Methods. A challenging way to face enantioseparation issues is to identify, with a unique chiral column, the best combination of eluent variables able to improve the degree of complementary chiral selector-analyte interactions. Barely considered aspects concerning the impact of the mobile phase components on the overall enantiorecognition process, by employing both low- and high-molecular weight chiral selectors, are discussed.

Results. With the use of a chiral ligand-exchange chromatography system operating with the O-benzyl-(S)-serine as the eluent chiral mobile phase additive, the best enantioresolution is provided by Cu(II) formate and Cu(II) fluoride, for the majority of the selected hydrophobic aminoacidic analytes.
The possibility to run the analyses with all the elution regimes allowed to identify the NP-based eluents as the most suitable, for the simultaneous diastereo- and enantioseparation of four non-steroidal FXR agonists, when analyzed with an anion-exchange-based chiral stationary phase incorporating a quinine derivative as the chiral selector.
In the enantioseparation of six anti-breast cancer purine derivatives, with a cellulose tris(3,5-dimethylphenylcarbamate)-based chiral stationary phase, relevant improvements of the overall chromatographic performance is obtained with the use of ''non standard'' solvents as constituents of the eluent mixture. The reliance on otherwise detrimental solvents was made possible due to the immobilized nature of the enantioresolving agent, which confers an universal

solvent compatibility to this kind of stationary phase and opens the way to previously unexplored enantioselectivity profiles.

Conclusions. The mobile phase should not be regarded as a passive transporter of the analytes along the column, but it is rather an essential component inherently involved in the enantioselective chiral selector–selectand association mechanism at multiple levels**.**

RESVERATROL-LOADED NANOCARRIER FOR INNER EAR DELIVERY

[1]U. M. Musazzi, [2]I. Youm, BB. [2]B. B. C. Youan

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Purpose. The aim of the work is to develop resveratrol-loaded nanocarriers (RES-NCs) for inner ear delivery and to evaluate in vitro toxicity on cochlear cell lines.

Materials. and methods RES-NCs are prepared by solvent-diffusion technique without surfactant. Resveratrol (RES), poly(D,L-lactide-co-glycolide) (PLGA) and poly(ε-caprolactone)–poly(ethylene glycol) diblock (PCL-PEG) are mixed in different ratios, dissolved in acetone and added dropwise to aqueous phase under constant stirring (acetone/water ratio 1/10). RES-NCs are washed and freeze-dried. Box-Behnken design (BBD) is used to study influence of RES-NCs composition on Z-size, PDI, Zeta-potential, drug encapsulation efficiency (EE%) and ratio between RES-NCs Z-size before and after freeze-drying (Sf/Si). In order to increase RES-NC stability during freeze-drying, lactose, mannitol, sucrose and trehalose are tested at different concentrations (1%, 5%, 10%, 15%, 20%w/v). Finally, MTS and LDH assays are carried out to check RES and Blank NCs toxicity after 24h incubation on two different cell lines: an organ of Corti model (HEI-OC1) and a stria vascularis one (SVK-1).

Results. BBD model is validated since all experimental responses fit with predicted values. Checkpoint analyses (bias NMT 10%) and Montecarlo simulation (response defect values NMT 10%) show good robustness in model capability to predict RES-NCs properties. The optimal formulation (desirability: 0.86), made of 7.4mg of RES, 3mg of PLGA and 5.3mg of PCL-PEG, correspond to Z-size of 136.2nm, PDI of 0.127, Z- potential of -26.80mV, EE% of 100.09% and Sf/Si of 3.30. All cryoprotectants increase RES-NCs stability during freeze-drying, disaccharides are more effective than mannitol. However, only trehalose in concentration higher than 15%w/v maintains Z-size and PDI in model space. In vitro toxicity studies show that RES can decrease cell viability only at concentration higher than 500µM, whereas blank NCs are toxic on HEI-OC1 in concentration more than 800µg/mL.

Conclusion. RES-NCs are successfully prepared by emulsion-diffusion technique and optimized by BBD. Moreover, threalose at 15%w/v guarantees RES-NCs stability during freeze-drying process.
Finally, in vitro studies show that RES and NCs are not toxic for cochlear cell lines in concentration lower than 500µg/mL and 800µg/mL respectively**.**

Posters

SYNTHESIS AND CHARACTERIZATION OF GOLD NANOPARTICLES

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Purpose. The aim of this study was to synthesize gold nanoparticles (AuNPs) to use in therapeutics and diagnostics for different diseases and in particular for cancer.

Methods. NPs were synthesized by the reduction of Au^{+3} (HAuCl4) to Au^0 using four different methodologies. In the first method, AuNPs were prepared by reduction of $HAuCl_4$ using citrate as both reducing and stabilizing agent [1]. In the second method, citrate acted as reducing agent while an aqueous solution of gelatine (30% w/v) was used as stabilizing agent [2]. Gelatine creates a network which limits ionic diffusion to restrict NP growing. With this method, three batches were prepared adding the gelatine solution in different predetermined time. In the third synthesis, AuNPs were obtained mixing an aqueous solution of $HAuCl_4$ with an aqueous Pluronic P84 solution [3]. The copolymer was used as both reducing and stabilizing agent. In the last method, glycerine was employed to reduce Au^{3+} to Au^0. Photon Correlation Spectroscopy (PCS), also known Dynamic Light Scattering (DLS), was used to characterize AuNPs in terms of size and stability. Particle size was expressed as mean hydrodynamic diameter ± standard deviation.

Results. The four methodologies yielded NPs characterized by different dimensions. NPs obtained using citrate showed a mean diameter of 16.59±1.79 nm. After thirty days of storage at room temperature, the mean particle size slightly increased (19.30±1.63 nm) and a new particle population (38.60±1.41 nm), accounting for 2.65% of the total, was observed. The use of gelatine as stabilizing agent did not change the NP mean size and stability in comparison with the first method. However, when gelatine was added before starting the reaction, reduction to Au^0 lasted 20 days. AuNPs obtained using Pluronic P84 showed three populations (18.66±9.06 nm, 91.96%; 127.53±1.71 nm, 7.63%; 210.4±4.7 nm, 0.77%) while glycerol yielded just two populations; the first one (91.7%) of 17.76±1.75 nm and the second (8.3%) of 122.03±0.98 nm. After ten days, AuNP prepared with the last 2 methods described showed no or minimal particle size variation.

Conclusion. AuNPs prepared using the different methodologies are characterized by different particle size and are stable upon storage at room

temperature for at least 10 days. The different methods, avoiding the use of organic solvents, can be considered as green synthesis.

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BIOACTIVE PHYSICAL CHITOSAN-BASED GELS FOR WOUND HEALING

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Purpose. Chitosan (CHI) is an hydrophilic polymer widely studied in the biomedical field due to its characteristics of biocompatibility and biodegradability. In this work we investigate the potential of new CHI-based physical gels, alone or loaded with active agents, namely herbal extracts or growth factors, for wound healing.

Methods. CHI powder was sterilized in autoclave and it was dissolved at the concentration of 2% w/w, in acetic acid 0.1 M; the resulting dispersion was sonicated and placed under vacuum for 48h. CHI gels were differently loaded depending of the considered active. Gels loaded with growth factors as PDGF and FGF-α were prepared by co-dissolution of the growth factors with CHI in acetic acid 0.1 M. Herbal extracts as Centella asiatica (CEN) or Echinacea angustifolia (ECH) were added to the preformed gel. Thermal and rheological analysis were carried out to highlight the differences due to the heat treatment and the viscoelastic properties of gels. The effects of the different gels on cell viability, proliferation and migration were investigated on human foetal foreskin fibroblasts (HFFF-2). Finally, the effect of the different gels was investigated on C57BL/6J mice using an experimental model of pressure ulcer.

Results. The gel analysis by differential scanning calorimeter (DSC) showed that, the thermal treatment modifies the chemical and physical characteristics of the CHI powder. Indeed, the formulations with non-autoclaved CHI showed a rheological behavior typical of a viscous entangled solution, while gels prepared from the autoclaved CHI have a typical gel behavior. Both the powders showed an endothermic peaks at ~ 100°C associated to the evaporation of water but they differ both for area and position, indicating that the macromolecules differ in their strength of water-polymer interaction. The loading of actives into the gel did not affect the rheological properties of the formulations which remained those of a typical gel. In vitro tests on HFFF-2 showed that CHI gels were not toxic and increased cell proliferation and

migration. Finally, the presence of PDGF, FGF-α, CEN or ECH enhance cells migration and proliferation without affecting the vitality of fibroblasts. In vivo, CHI gel enhanced wound healing compared to untreated mice. This effect was higher when using CHI gels containing active compounds. Further in vivo studies are on-going to understand what is the active, or mix of the active with the highest effect to accelerate wound healing.

Conclusions. The thermal treatment of CHI changes the physical and chemical characteristics of the powder of CHI ensure their sterilization. The sterilization of the powder also changed the rheological properties of the CHI solution that have characteristics more close to a gel; this should favour the permanence of the formulations in the wounds. In vitro studies showed that CHI gels are non-toxic and these formulations induce cells proliferation and migration especially when loaded with active compounds. Preliminary studies in vivo have confirmed the regenerative properties of the formulations and the high potential as new products for the medication of wound, i.e. pressure ulcers.

TOXICITY OF CADMIUM ON SERTOLI CELLS FUNCTIONAL COMPETENCE: AN *IN VITRO* STUDY

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Purpose. Cadmium (Cd), an ubiquitous environmental pollutant mainly used for industrial purposes, is highly associated with reproductive toxicity. Sertoli cells (SC), by providing an appropriate microenvironment for the development of germ cells, play a pivotal role on spermatogenesis regulation (Geoffroy-Siraudin et al. 2012). Aim of our investigation was to assess the effects of Cd on high mammalian SC viability and function.

Methods. Porcine pre-pubertal SC were isolated, according to previously established methods (Fallarino et al. 2009) and treated with 3 different concentrations (5-10-15 µM) of Cd chloride. Cd uptake was assessed by ICP-OES analysis after extraction in 1M HNO3 solution.

Results. The evaluation of SC function in terms of inhibin B and anti-Müllerian hormone (AMH) secretion showed a significant decrease in all SC treated conditions respect as compared to SC control. On the contrary, evaluation of the FSH-R integrity on SC surface, in terms of 17-β-estradiol production under FSH stimulation, showed no difference between SC control and 5 µM Cd treated SC monolayers in comparison to 10 and 15 µM Cd treated SC monolayers, where FSH-R was impaired. In addition, the apoptotic test showed a significant increase of early (ANNEXIN V-/Propidium Iodide+) (AV-/PI+) and late apoptotic cells (AV+/PI+) in all Cd treated SC conditions in comparison with SC control. Cd uptake and cell accumulation correlated perfectly with toxicity data.

Conclusions. Our data demonstrate that Cd, even at low dose, exerts toxic effects on Sertoli cells function possibly adversely affecting the spermatogenesis.

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ENHANCED PROTECTION AGAINST OXIDATION ON HYDROPHOBIC VITAMINS BY NANO-ENCAPSULATION

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Purpose. The success of liposomes as carriers of hydrophilic and hydrophobic substances has been reflected in a number of liposome-based formulations. The use of liposomes has many benefits, including improved penetration of active ingredients, selective transport, longer release time, greater stability of active substances and high biocompatibility. Here a successful oxidative damage prevention of hydrophobic vitamins (retinyl palmitate and α-tocopherol) by liposomes encapsulation has been proposed and tested.

Methods. Reduction of liposomes vesicles size to the nano-levels has been performed by high shear fluid processor M-110EH by Microfluidics Corp. providing a fine and homogenous dispersion, sterilizable by filtration. This study shows excellent chemical stability of both vitamins encapsulated in liposome-formulation proposed.

Conclusions. Nano-encapsulation has shown to be effective in protecting hydrophobic vitamins, well known as sensitive to oxidative degradation. Results show this protection to be effective either at room temperature and at 40°C.

OPTIMISATION OF A FILM-FORMING MUCOADHESIVE VEHICLE TO BE SPRAYED ON THE BUCCAL MUCOSA

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Purpose. To optimize a liquid film-forming vehicle to be sprayed without propellant gas on the buccal mucosa.

Methods. Stock solutions of mucoadhesive film-forming materials were prepared by dissolving Polyvinyl alcohol (PVA) 5% w/w and Na hyaluronate (HA) 2% w/w in distilled water and polymethacrylate (Eudragit® S100) 5% w/w in NaOH 1 M. All solutions were adjusted to pH 7.2 ± 0.05. Stock solutions were employed as such or mixed in predetermined w/w ratios to obtain the significant points (10) of a simplex lattice design.
The samples were characterized for viscosity at 25°C (storage temperature) and at 37°C (application site temperature).
Time for filming was measured by spraying with a no-gas-pump a metered amount of each sample from a distance of 6 cm on a film prepared by drying a 8% w/w porcine gastric mucin dispersion in pH 6.4 phosphate buffer, chosen to mimic the pH of the buccal environment. The sample was placed in a thermostated (37°C) shaking (60 rpm) water bath and film formation was assessed by visual inspection. For spray area evaluation each sample was delivered as above described on filter paper; the wet area was cut, dried and weighed and the weight was compared to that of a filter paper disc of known area.
Experimental data were treated with a statistical package, according to the experimental design chosen.

Results. The best fit model of all response variables (viscosity, time for filming, spray area) was the special cubic one.
The comparison of the experimental values of a given variable with those calculated by the model proved that the model was always predictive ($p<0.05$).
The superimposition of the contour plots of each response variable enabled us to identify the region of optimal composition of the vehicle, characterized by lowest viscosity at 25°C, highest viscosity at 37°C, lowest time for filming, highest surface area. The optimized composition was: PVA 2.35% w/w, Eudragit® S100 2.35% w/w, HA 0.12% w/w.

Conclusions. The liquid in situ film-forming vehicle of optimized composition represents a promising candidate for the administration of drugs intended to be sprayed by a no-gas pump on the buccal mucosa.

A PROTOTYPE OF AIRLESS SPRAY-GUN PLANT FOR MICROINCAPSULATION OF BIOACTIVE PRINCIPLES

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Purpose. To develop, assemble and test a prototype of a novel production plant, suitable to produce microparticles (MPs) processing highly viscous feed solutions (FSs).

Methods. The prototype was composed of commercial air compressor, piston pump and airless spray-gun and by customized air-treatment section, timer, rotating base, hood and filtration section. We performed a preliminary prototype parameter setting i.e. nozzle's dimension, nebulization timing, CaCl2 concentration in the gelation fluid. Prototype throughput (1 to 5 L) and the range of practicable FS viscosities were assayed. Rheological analyses were performed on FSs containing alginate up to 5% w/v. A set of four batches was prepared in order to characterize the MP features, in term of particle size and distribution, flowability, swelling, encapsulation efficiency and release.

Results. According to the qualitative scoring, the large nozzle was more suitable to nebulise FSs at higher alginate concentration than small nozzle. Conversely, the small nozzle performed better than the large one to process FSs with alginate concentration of 2%. Only at the highest degree of viscosity, corresponding to 5% w/v of alginate, the processing was not technically possible. Among the concentrations of CaCl2 assayed, 15% w/v was recognized as the more versatile. The prototype appears to be convenient and to grant a high yield starting from 2 L of FS. The flow behaviour of the FSs assayed can be satisfactorily described with the Carreau-Yasuda equation. The throughput Q begin to decrease significantly for FSs with an alginate concentration above 3% p/w with a profile that can be described by the following stretched exponential relation:

$$Q = 437 \exp\left(-(0.18\ c)^{7.2}\right)$$

MP morphology was irregular, with crumpled shape. The angle of repose indicates a good flowability and the release studies showed a good gastroresistance and a potential in prolonged release applications.

Conclusions. The novel prototype of production plant is suitable to process large amounts (2 L or more) of FSs, characterized by a high viscosity, to produce MPs.

MODULE ASSEMBLY TECHNOLOGY TO MANUFACTURE EFFERVESCENT TABLETS IN A NON DEHUMIDIFIED PRODUCTION PLANT

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Purpose. Dome Matrix® platform can combine 2-3 drugs into a unit oral dosage form based on module assembly strategy, to simplify complex therapies. As the assembly size increases, dispersion in water before administration may be desirable. Here, excipient modules were studied for promoting assembly dispersion by effervescence obtained by introducing the reagents or drugs in separated modules.

Methods. Citric acid and sodium hydrogen carbonate were tableted separately, at increasing percentages, by direct compression of mixtures also containing Avicel®, Explotab® and Mg Stearate as lubricant. 7.4 mm Dome punches were used. Effervescence of assembled modules was quantitated by measuring the CO2 developed in 20 ml of water (Bernard calcimeter). Then, a drug module of pyrazinamide (80% w/w) was manufactured to study the effect of effervescence on assembly disintegration and drug dissolution in vitro.

Results. Direct compression of effervescence reagents was feasible up to 70-80% (w/w) content in each module, but cohesion became poor above 50-60% (w/w) and friability increased, particularly for the acid module. High percentages of citric acid made the module more prone to erosion than disintegration. The amount of CO2 produced from the reaction in water of one acid module and one NaHCO3 module depended on reagents' molar ratio, being higher in excess of acid. Moreover, the higher the reagents' concentrations, the more intense the turbulence given by effervescence. Tableting delayed the beginning of the effervescence reaction in comparison with that given by equivalent amounts of the pure reagents in powder form. The insertion of the pyrazinamide module between the reactive ones did not interfere with effervescence development. As pyrazinamide is highly soluble in water by itself, drug dissolution was not affected by effervescence, being completed within 5 minutes.

Conclusions. Dome Matrix® technology allowed to manufacture an effervescent oral dosage form in which the reactive agents were physically separated in different modules. Modularity could facilitate the manufacturing process and improve product stability compared to conventional effervescent systems where acid and CO2-generating agent are intimately mixed. The effervescent assembled system could be proposed to formulate a pediatric dosage form when many drugs at high doses have to be administered orally (e.g. in tuberculosis).

DESIGN OF ALGINATE BASED AEROGEL FOR NONSTEROIDAL ANTI-INFLAMMATORY DRUGS CONTROLLED DELIVERY SYSTEMS USING PRILLING AND SUPERCRITICAL-ASSISTED DRYING TANDEM TECHNIQUE

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Purpose. To study the possibility to use prilling and supercritical antisolvent extraction (SAE) as tandem technique to obtain alginate based beads loaded with NSAIDs drugs, as ketoprofen (K) and ketoprofen lysinate (KL) for oral administration. To study the effects of this technique in terms of particle morphology, drug solid state properties and drug release rate in gastrointestinal tract.

Methods. Alginate beads were produced by prilling processing different drug/polymer feed solutions using a 600 μm diameter nozzle and setting volumetric flow rate and vibration at appropriate values to obtain hydrated beads in narrow size distribution. Hydrated beads were gelled using a 0.3 M CaCl2 aqueous or ethanolic solution. Calcium alginate beads produced in ethanolic solution were directly dried by SAE whereas beads obtained in aqueous solution required a pretreatment with ethanol to displace water and then dried. Beads size distribution, morphology and inner structure were determined by light laser scattering (LLS) and scanning electron microscopy (SEM). Solid state studies on loaded beads were performed using differential scanning calorimetry (DSC) and X-ray. Finally, drug release studies were conducted in sink conditions with a USP dissolution apparatus II.

Results. Drug/polymer ratio and gelling conditions influenced mean diameter and morphology of the beads. SAE drying preserved nanofibrous alginate network and particle shrinkage was reduced. Encapsulation efficiency was good (> 58%) for K loaded beads. Cross-linking alginate in ethanol lead to crystal clusters of K embedded in beads polymer matrix whereas cross linking in aqueous solution produced nanometric particles of amorphous K inside beads. Dissolution profiles of K loaded beads dried by SC-CO2 presented an enhanced burst effect in simulated gastric fluid (SGF), especially for aerogels obtained from aqueous gelling solution.

Conclusion. Prilling and SAE tandem technique allow production aerogels with nanoporous structure and narrow size distribution with good drug loading. Nanoporous structure of the bead matrix as well as amorphous state of the encapsulated drug enhance dissolution rate of K, especially in SGF. These properties are very useful in order to obtain a rapid onset formulation for BCS class II drugs.

INTERACTIONS BETWEEN ATENOLOL AND POLYSACCARIDES BY H1-NMR AND HATR

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Purpose. As a part of a project aiming at collecting new insights to be used in the development of controlled drug delivery systems, the goal of the present work was to investigate and rationalize the interactions between a model drug containing basic groups, such as atenolol (ATN), and biocompatible model polymers presenting different chemical functions.

Methods. Chitosan (CH) (DD: 95%), chondroitin sulphate (CS), sodium alginate (ALG) and λ-carrageenan (λ-CAR) were selected as polymers. The study of interactions was carried out by HATR spectroscopy and H1-NMR analyses. HATR experiments were conducted by moistening ATN-polymer blends with buffers at two pH values: 4.5 and 7.4. H1-NMR analyses were carried out on the solutions of ATN and polymers in D2O and D2O/CD3COOD 1% v/v.

Results. Significant variations in HATR spectra of ATN-polymers mixtures relevant to the peaks associated with stretching of amine (C-NH), hydroxyl (C-OH) and ether groups (C-O-C) and in-plane deformation of hydroxyl of ATN were observed at both pHs. Wavenumber shift and reduction of intensity of these signals were observed for all polymers while strong C-OH, C-O-C band widening were noticed only for ATN-ALG and ATN-λ-CAR mixtures.
H1-NMR spectra recorded at pH 7 showed significant variations for ATN-polymer (CS, ALG, λ-CAR) solutions. The shifts mainly involve the hydrogen atoms of the ATN lateral chain where amine and hydroxyl functional groups are present, supporting HATR evidences. The ATN-CS mixture exhibits the widest δ variations (Δδ 0.43) suggesting the presence of both hydrogen and ionic bonds between the drug (amine pKa: 9.6) and polymer carrying anionic sulphate groups. Moving to ALG and λ-CAR, H1-NMR spectra of ATN showed a progressive reduction in δ variations (Δδ 0.23 and Δδ 0.2, respectively) suggesting a weaker interaction.

Conclusion. These preliminary studies support the presence of interactions between ATN and the polymers both in the solid state and in solution. H1-NMR data clearly exhibit differences in the ATN interactions as a function of the

polymer functional groups. Such interactions mainly involve ATN amine and hydroxyl groups via ionic and hydrogen bonding.

ON THE POSSIBILITY TO BIOENGINEER SKIN ON CHICK CHORIOALLANTOIS

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Purpose. To evaluate the possibility to engineer skin on the chicken chorioallantoic membrane (CAM) starting from de-cellularized de-epidermized human dermis (DED) [1] and immortalized keratinocytes with the aim of replacing animals in short-term investigations in dermatology.

Methods. Immortalized human keratinocytes (Hacat) [2] were cultured on DED for 3 days. Fertilized chicken eggs were incubated and, on day 7, circular DED samples (6 mm) were implanted on the CAM after provoking an ectodermal epithelium lesion with a teflon punch. On day 12 the xenografts were excised together with the surrounding CAM, the tissue fixed in 4% paraformaldehyde, paraffin-embedded and cut with a microtome to obtain 5-10 μm slides. Staining was performed with hematoxylin and eosin. Alternatively, whole dehydrated samples were imaged by scanning electron microscopy (SEM).

Results. Already 2 days after transplantation, the skin grafts were invaded by the CAM erythrocytes and neo-endothelium was present. The presence of nucleated chick erythrocytes confirmed that the DED was nourished by the host blood. Epidermal growth factor (EGF) [3] accelerated and improved neoangiogenesis in the CAM and implanted DED. On day 12, SEM observation of the DED cultured with Hacatrevealed keratinocyte stratification and the initial development of a multilayered epithelium.

Conclusion. Our results indicate that it is possible to use the revascularized DED to support the formation of an epidermis provided with stratum corneum. The future goal of this project is to establish the epidermal barrier using primary epidermal cells and test the barrier function by examining the permeability to various chemical compounds.

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IN VITRO DIGESTION STUDY ON SOLID LIPID NANOPARTICLES AND NANOSTRUCTURED LIPID CARRIERS DELIVERING INSULIN

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Purpose. The research was aimed to compare the digestion of two different kind of lipid nanoparticles (NP): Solid Lipid Nanoparticles (SLN) and Nanostructured Lipid Carriers (NLC), delivering insulin-chitosan complexes.

Methods. Lipid Nanoparticles were prepared by high-shear homogenization of lipids (Precirol® for SLN and a mixture of Precirol® and Miglyol®/squalene for NLC) and an aqueous phase containing Tween®80 and Pluronic®F127, as surfactants. The NP were loaded dissolving an insulin-chitosan complex in the aqueous phase. Lipase and co-lipase digestion was performed on both loaded and unloaded lipid carriers. Briefly, 150 µl of SLN/NLC were incubated at 37°C with a mixture of lipase (2000 U) and co-lipase (30 µg), buffered at pH 7.4. The reaction was monitored by sampling each 15 minutes until 1 hour and was stopped by addiction of HCl 0.01 N. The entity of digestion was evaluated by extraction and quantification of free fatty acids. To assess if any variation in digestion profile could occur because of physical differences among them, SLN and NLC were characterized also in term of particle size and zeta potential. Mucoadhesive properties were also evaluated.

Results. Among the unloaded nanoparticles, SLN were the most digested, followed by Miglyol-NLC, whereas digestion for squalene-NLC was close to zero. The loading with insulin-chitosan complex resulted in a reduction of NP digestion, with the exception of squalene-NLC, whose digestion showed a slight initial burst effect. This could be attributed to a change in lipid distribution, where squalene could be conceivably pushed to the surface of the NP. Loading induced a slight increase in particle size for all NP and even more important modifications in Z-potential, that became strongly positive for SLN and Miglyol-NLC. The positive charge was likely due to the presence of chitosan on the surface of these NP, that however did not acquired mucoadhesive properties.

Conclusions. In order to obtain nanocarriers quite resistant to enzymatic degradation, more attention should be paid to NLC, that resulted less digested than SLN. Furthermore the choice of the liquid lipid, as well as the structure of

the final assembling of loaded nanocarriers, emerged to be fundamental to modulate digestion rate.

TECHNOLOGICAL CHARACTERIZATION OF FAST DISSOLVING FILMS MADE OF MALTODEXTRIN

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Purpose. Oral dispersible films are solid dosage forms which dissolve and/or disintegrate within less than 1 minute in the oral cavity avoiding the need of drinking or chewing. Films with suitable mechanical properties are obtained by addition of a plasticizer, namely glycerine, to maltodextrins.
However, the main critical aspect is that these films tend to become less flexible over time and the stiffening of the film may affect stability and handling of the product.
The objective of this work is to stabilize the mechanical properties of the films over time.

Methods. A study performed by ATR-FTIR spectroscopy aimed to evaluate the possible interactions between maltodextrins and BTY28, a novel water insoluble excipient.
Five placebo formulations were prepared, containing increasing percentages of BTY28 (1-20% w/w) and the performances of the formulations obtained were compared with those of a film free from this additive. In particular, the films were studied in terms of time of disaggregation and tensile properties.
The most suitable formulation was chosen to prepare orodispersible films containing, as model drug, Diclofenac Epolamine. In this case, the films obtained were characterized by determining drug content, impurities and dissolution profile, in addition to the properties reported for placebo films.
The stability of the films was followed over a six months period on samples stored, both in normal and accelerated conditions, in accordance with the ICH guidelines.

Results. A comparison between the ATR-FTIR spectra of binary mixtures of maltodextrins and BTY28 highlighted that BTY28 was able to modify the three-dimensional organization of maltodextrins by forming hydrogen bonds.
As expected the MDX films stiffened in the first three months of storage; the addition of BTY28 in the 3-5% range permitted to obtain dosage form with good mechanical properties, which resulted stable over time.

Moreover, the addition of this additive didn't affect dissolution and disaggregation of the films loaded with Diclofenac Epolamine, and its chemical stability.

Conclusions. The use of BTY28 permitted to improve the stability of mechanical properties of film made of maltodextrins and therefore this formulation could be advantageously used for the production of fast dissolving film.

THE ROLE OF THE CONFORMATIONAL PROFILE OF POLYSACCHARIDES ON SKIN PENETRATION: THE CASE OF HYALURONAN AND SULFATES THEREOF

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Purpose. To provide a relationship between diffusion processes of hyaluronans (HA) through the human epidermis and their physico-chemical properties. In particular, the present work focused attention on the impact of molecular weight, conformation and polarity on the skin penetration ability of these polysaccharides.

Methods. Low- and medium-molecular weight HA and the corresponding derivatives at two degrees of sulfation (HAS) were tested. The transdermal penetration of HA polymers was experimentally determined by the Franz diffusion cell method using human epidermis as membrane The localization and the diffusion pathway of HA through human epidermis were followed by confocal laser scanner microscopy using a FITC-labeled HA. The possible relationship between the HA conformation and the ability to permeate the human epidermis was studied by in silico molecular dynamics (MD) simulations.

Results. The in vitro experiments evidenced that HAs cross the epidermis mainly through a transcellular route, the higher the molecular weight, the higher the permeated amount. The epidermis resulted more permeable to the HAS than HA and the permeated amount increases with the degree of sulfation. The molecular dynamics study evidenced how the observed permeation behaviour can find compelling explanations in the conformational profiles since the permeation increases with the capacity to assume extended and flexible structures, as encoded by the values of radius of gyration: the highest the value of radius of gyration, the highest the permeated amount.

Conclusions. This work demonstrated that the main parameters that rule the diffusion of small molecules through the skin are not limiting factors for the skin permeation of hyaluronans. These molecules have a great affinity for

corneocytes and likely cross human epidermis mainly through a transcellular route, rather than the intercellular one. Moreover, the molecular dynamics study revealed that a simple parameter such as the radius of gyration, which is an expression of the polymer folding, could be used in predicting the extent of the permeation of a homogeneous family of polysaccharides through human skin.

PLANT VIRUS NANOPARTICLES BASED ON TOMATO BUSHY STUNT VIRUS: A MULTIFUNCTIONAL TOOL FOR NANOBIOTECHNOLOGY

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Purpose. Viral nanoparticles are molecular cages derived from the assembly of viral structural proteins. Recently, plant viruses derived nanoparticles have received much attention as molecular tools in different technological fields. The aim of the study consists in the development of an efficient and versatile system for VNPs production in Nicotiana benthamiana based on Tomato bushy stunt virus (TBSV).

Methods. Standard molecular biology techniques have been used to construct chimeric virus nanoparticles (CVNPs) displaying specific tag peptides fused to the viral coat protein. Several CVNPs have been obtained and analyzed by: i) transmission electron microscopy to verify the correct assembly, ii) RT-PCR and sequencing to evaluate genomic stability and iii) SDS-PAGE and Western blotting for characterization at proteomic level. Moreover, suitable protocols have been realized for the entrapment of little exogenous molecules in the TBSV inner core and for the in vitro chemical derivatization of the viral outer surface.

Results. Genetic modification of the viral coat protein gene has allowed protein fusions up to 56 amino acids in length, leading to correctly assembled CVNPs that display the peptide of interest on the virus outer shell. The system has shown an extreme versatility also regarding chemical modifications: N-hydroxysuccinimide ester based chemistry has been successfully employed to biotinylate lysine residues on the TBSV surface. Finally, a method has been developed to entrap ethidium bromide molecules inside the VNPs cavity by reversible opening of virion gated pores, a structural transition induced by controlling physicochemical parameters such as pH and concentration of metal ions.

Conclusions. The reported results evidence the fine tuning and the characterization of a TBSV based nanovector that can be used for the encapsulation of small molecules and on which a predictable and programmable genetic and/or chemical engineering can be performed.

NEW CXCR4 INHIBITORS COUPLED WITH LIPOSOMES INHIBIT LUNG METASTASES DEVELOPMENT

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Purpose. Development of liposomes functionalized with peptides antagonist of CXCR4 receptor, such as a potential therapeutic systems in the prevention of lung metastases.

Methods. Two different anti CXCR4 peptides were synthesized, respectively pepR and pepS, with a sequence of three amminoacidis common to the amminoterminal chain of CXCL12, the CXCR4 ligand, and the viral secreted chemokine vMIP-II. Liposomes composed of DPPC/Chol/DSPE-PEG or DPPC/Chol/DSPE-PEG-Mal were prepared. Then, the peptides were conjugated with liposomes containing DSPE-PEG-Mal. The mean diameter, the size distribution and the zeta potential (ζ) of liposomes, with and without CXCR4 antagonists, were determined by photon correlation spectroscopy and by means of a Zetasizer Nano Z, respectively. Selected formulations were tested on several cancer cell lines and in vivo in an experimental model of lung metastases (C57/BL).

Results. The mean diameter of conjugated and non-conjugated liposomes was found to be between 130 nm and 140 nm, with a narrow size distribution (P.I.≤ 0.1). Moreover, after conjugation of anti-CXCR4 peptides with liposomes, compared with the unconjugated liposomes, showed a significant charge shielding, as demonstrated by the reduction of the ζ from -23 to -6 mV. In the human renal cancer cell (RXF393), anti-CXCR4 peptides, naked or conjugated with liposomes, significantly inhibit cell migration. In vivo, mice treated with pepR-conjugated liposomes showed greater reduction of pulmonary metastases, if compared to mice treated with the same dose of naked pepR.

Conclusion. Liposomes conjugation to an CXCR4 antagonist could represent a new strategy to prevent or reduce the onset of lung metastases.

INSULIN POWDERS FOR PULMONARY ADMINISTRATION

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Purpose. The aim of this work was to produce and characterize insulin respirable powders for the treatment of diabetes disease. The powders were prepared with or without chitosan in order to obtain an immediate or modified release of the peptide.

Methods. Insulin microparticles containing recombinant human insulin (Ins-SD) were manufactured by spray drying an acid solution (pH = 4.0). A spray-dried powder was also prepared with the addition of chitosan (InsChit-SD). The ratio between insulin and chitosan was 98:2.
Afterwards, both powders were characterized in terms of drug loading, morphology by Scanning Electron Microscopy (SEM), particle size by laser diffraction, and aerodynamic particle size by Next Generation Impactor (NGI). RS01® device was employed to aerosolize microparticles at 60 l/min. In vitro dissolution test was carried out using Franz's cell apparatus. Statistical analysis of the dissolution curves were performed calculating similarity factor (f2).

Results. Ins-SD and InsChit-SD microparticles were successfully manufactured by spray drying and the yield of process was higher than 60%. SEM images of Ins-SD showed microparticles roundship with corrugated surface. No morphological differences were observed when chitosan was added to the formulation. Both insulin powders presented dV50 less than 5 µm and high respirability values. In particular, Fine Particle Fraction was equal to 78.0% and reached 92.1 % when chitosan was added. Mass Median Aerodynamic Diameter value was equal to 2.04 µm and 1.21 µm for Ins-SD and InsChit-SD respectively (n=3). InsChit-SD showed slower dissolution profile than Ins-SD ($f2<50$).

Conclusions. Insulin spray-dried microparticles can be produced by spray drying an acidic solution of the peptide. The presence of chitosan decreased the drug dissolution rate and improved the aerodynamic performance.

MULTIFUNCTIONAL MICROFIBRES BY MICROFLUIDICS FOR TISSUE ENGINEERING APPLICATIONS

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Purpose. The work describes the fabrication of cell containing multifunctional microfibres which have great potential for applications in drug release formulations and tissue engineering scaffolds providing cell structural support and immunoisolation. Recent findings demonstrated that the co-encapsulation of cells and drug delivery systems offered a rational alternative to reduce the foreign body response to the scaffolds, improving the cell viability and functional competence, therefore enhancing the effectiveness of the tissue engineering construct. Based on the promising results showed by this strategy, we designed a microfluidic dispersing chip permitting the production of microfibres for the simultaneous embedding (within the fibre matrix) of living cells together with other particulate or soluble components such as the extracellular cell matrix.

Methods. Alginate microfibres were produced using glass-based microfluidic chips fabricated by a photolithography-wet etching procedure. A sodium alginate solution and two sodium alginate suspensions were delivered via the three inlets. The two suspensions contained different amounts (10–40 mg mL-1) of either drug delivery systems (previously prepared) or cell suspensions. The output from the outlet of the two chips was transferred via a Teflon microtube into a BaCl2 solution (0.5–3.0%, w/v) where the alginate flow stream was gelled to produce the final Ba-alginate consolidated microfibres. The produced microfibres were examined by an optical stereomicroscope. The viability of entrapped cells was assessed using a double staining method with propidium iodide (PI) and calcein-AM before and after encapsulation in alginate microfibers.

Results. The key parameters, which critically influence the formation of microfibres and their geometries, were firstly identified by a classical intuitive approach COST (Changing One Separate factor a Time). In particular, their effects on the microfibre diameter were investigated, which are directly associated with their functionalities relating to the implantation site, the nutrient

availability and diffusion/transport of oxygen and secretory products. The interplay between the alginate solution concentration, pumping rate and gelling bath concentration in controlling the diameter of the produced microfibres was investigated with a statistical approach by means of a ‘‘design of the experiments’’ (DoEs) optimization and screening. In the study, the microfiber preparation protocol developed was evaluated with human primary mesenchymal stem cells and lipospheres or microparticles. The fluorescence photomicrograph recorded immediately after the microfibre preparation shows that the cells maintained a very high viability (>95%), indicating that the presented preparation strategy is highly biological compatible and suitable for the encapsulation of primary cells.

Conclusions. A simple, cost-effective, well-controlled and biological compatible process has been developed for the production of uniform alginate microfibres, with controlled size and content. We demonstrated that the fibre diameter is controllable with the highly uniform diameter distribution along the entire fibre length. The key advantages of the microfluidic fibre generation system are the versatility of size, little limitation in fibre length and possible incorporation of drug delivery systems and living cells.

BSAO IMMOBILIZED IN SELF-ASSEMBLED HYALURONIC ACID NANOHYDROGELS INDUCES CYTOTOXICITY ON MELANOMA CANCER CELLS

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Purpose. To develop new strategies for melanoma cancer treatment, bovine serum amine oxidase (BSAO), an enzyme that is able to produce antincancer species in situ, was covalently immobilized onto injectable, self-assembling nanohydrogels (NHs) based on cholesterol-graft-hyaluronic acid (HA-CH).

Methods. To produce NHs, cholesterol was linked to HA chains by a spacer, 4-bromobutyric acid, with a double step reaction. At first, cholesterol was esterified with 4-bromobutyric acid obtaining Br-butyric-cholesterol (CH-Br); the second step was represented by HA esterification with CH-Br.. NHs were produced by sonication for 25 min of the water suspension of HA-CH , using an ultrasonic bath sonicator. NHs were characterized in term of size, zeta-potential, morphology, storage, stability and citocompatibility. To accomplish covalent enzyme immobilization on NHs, carbodiimide chemistry was used. The activity of immobilized enzyme was checked in vitro (spermine assay) and on a model melanoma cell line (M14 cells).

Results. Amphiphilic HA-CH forms spontaneously in water spherical NHs characterized by narrow distribution size (120±20 nm), and negative zeta-potential (-40mV±0.08mV). BSAO was covalently immobilized on NHs (yield 20±2%) retaining the activity. Experiments were performed on human melanoma cancer cells and it was demonstrated that, when melanoma cells were treated with the immobilized enzyme, an increase of the cytotoxicity was observed.

Conclusions. HA-CH NHs are a suitable delivery system for proteins. BSAO covalently linked to NHs shows high activity and could represent an alternative strategy for the treatment of the melanoma.

OPTIMIZATION OF CHITOSAN/SERICIN DRESSINGS FOR THE DELIVERY OF PLATELET LYSATE IN SKIN ULCERS

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Purpose. Platelet lysate (PL) is obtained by platelet destruction by freeze-thawing starting from a platelet rich plasma sample in presence of an anticoagulant agent. It is recognized that PL in contact with skin ulcers is able to promote wound healing. The aim of the present work was the preparation and characterization of chitosan sponge-like dressings to be used for the delivery of PL to skin chronic wounds. Dressings contained sericin (Ser), a silk protein recognized as an excellent biomaterial for tissue engineering.

Methods. Chitosan (CS) sponge-like dressing were prepared by freeze-drying of a CS HMw (DD: 91%) (1568, Giusto Faravelli, I) glutamate solution containing Sericin (Ser) and Glycin (Gly) (Sigma-Aldrich, I). Three stock mixtures based on different CS, Ser and Gly concentrations (%w/w) were prepared: 1.5/0.5/0; 0.5/1/0 and 0.5/0.5/1. Such mixtures were then employed as such or mixed in predetermined w/w ratios to obtain the significant points (7) of a simplex centroid design. Dressings obtained by such mixtures were characterized for mechanical resistance, hydration propensity and viscous and viscoelasticity properties upon hydration. The response variables considered were: distance and work of fracture, viscosity at low and high shear rates, elastic modulus at 2 Hz frequency, buffer uptaken after 6h hydration. Experimental data were treated with a statistical package (Statgraphics 5.0,Statistical Graphics, Rocknille, MD), according to the experimental design chosen. The optimized formulation was loaded with PL and was investigated in vitro for proliferation and wound healing properties on normal human dermal fibroblast cell line (bHNDF,Promocell,G).

Results. The best fit model of all response variables was the special cubic one. The dressing with optimized composition loaded with PL was able to promote wound healing. In particular a synergic effect between PL and Ser was evidenced.

Conclusions. Dressings based on chitosan (CS) glutamate, Ser and Gly are promising candidates for the delivery of PL in the treatment of skin ulcers.

HYALURONIC ACID CAPSULES IN ALGINATE DRESSINGS FOR THE COMBINED DELIVERY OF PLATELET LYSATE AND VANCOMYCIN IN SKIN ULCERS

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Purpose. An ideal wound dressing should provide a quick wound healing with minimal discomfort for the patient. It should be able to remove exudate in excess without the loss of the moisture of wound bed. It is known that hyaluronic acid (HA) is involved in several mechanisms of the wound healing process. It improves wound healing of chronic wounds. Also sodium alginate (SA) is widely known for its healing and haemostatic properties. Recently platelet lysate (PL), a hemoderivative obtained from platelet rich plasma, has demonstrated to accelerate healing process. The aim of the present work was the preparation and characterization of HA capsules loaded with PL and incorporated in SA dressings containing vancomycin.HCl (VCM), as model drug.

Methods. Blank capsules were obtained by dropping 1 ml of 3% w/w HA (low MW, Bioiberica, S) 1 and 2% w/w CaCl2 aqueous solution into 10 ml 1% w/w SA (MV, Sigma-Aldrich, I) solution under gentle stirring. TIO2 was added to HA solution as colouring agent. Capsules loaded by PL were prepared by substituting 0.5 ml of water of HA solution with 0.5 ml of PL. VCM was dissolved into SA solution at 3 mg/ml concentration. Blank and loaded dressings were obtained by freeze drying SA solution containing HA capsules. Dressings were investigated for mechanical properties by means of tensile measurements and for hydration propensity employing PBS as model medium. Viscosity and viscoelastic behavior of hydrated dressings were determined by a rotational rheometer. VCM release from dressings was evaluated by means of a Franz diffusion cell. Wound healing properties of dressings were in vitro evaluated on human fibroblast cells (bHNDF,Promocell,G).

Results. Dressings developed show suitable mechanical and viscoelastic properties and are able to absorb PBS, medium mimicking wound exudate. In particular dressings prepared with 2% w/w CaCl2 solution show the best elastic and hydration properties. All dressings are able to release VCM and are

characterized by cell proliferation properties, due to the release of the bioactive substances of PL from capsules.

Conclusions. The dressings developed are promising candidates for the combined release of VCM and PL in skin ulcers.

STREAMING POTENTIAL AS ALTERNATIVE TECHNIQUE TO STUDY DRUG ADSORPTION ON SCLERA

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Purpose. Trans-scleral drug administration is considered a possible alternative approach to the intavitreal injections in the treatment of the diseases affecting the posterior segment of the eye. However, some drugs tend to be adsorbed into scleral tissue and this could affect the diffusion. Aim of the present work was to demonstrate the ability of the streaming potential to study the drug absorption on bovine and porcine sclera.

Methods. As model compounds two low molecular weight drugs (methylprednisolone sodium succinate – MPSS - and propranolol) and a protein (cytochrome C) were chosen. Streaming potential ($\Delta E/\Delta p$) was measured with an apparatus built in lab. An electrolyte solution was pumped across sclera sample (source: cow and pig; exposed area: 0.9 cm2), while the pressure (Δp) and voltage difference (ΔE) were monitored. The tissue was mounted in the cell and equilibrated with the solution for at least 30 minutes before starting the measure (measurement time: 30 minutes). The tested solutions (1, 5 and 9 mM for both MPSS and propranolol; 0.08 and 0.41 mM for cytochrome C) had the same ionic strength of the reference (10 mM NaCl in distilled water).

Results. Streaming potential has the same sign as the surface of a biological membrane and therefore it is possible to determine if a compound is adsorbed into a tissue by evaluating the change in the streaming potential values. The results obtained confirmed that both porcine and bovine sclera carry a negative charge, but bovine sclera has a higher surface charge density. MPSS determined an increase in the negative surface charge of the tissue, due to the negative charge carried, while both propranolol and cytochrome C shifted the charge towards positive values. This result indicates that all the model compounds were adsorbed on both bovine and porcine sclera, already in a millimolar concentration range; furthermore, the absorption (propranolol vs bovine sclera) is not completely reversible.

Conclusions. Streaming potential represents a convenient means to study the adsorption of drugs on biological tissues.

PhSeZnCl A NOVEL SELENIUM COMPOUND WITH GPx-MIMETIC ACTIVITY: FORMULATION AND IN VITRO CHARACTERIZATION

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Purpose. The aim of the present work is to formulate and characterize in vitro potentially respirable spray-dried microparticles (MP) of PhSeZnCl, a novel compound with GPx-mimetic activity, in order to improve the efficacy of the molecule and reducing the dose-dependent cytotoxicity.

Methods. PhSeZnCl was encapsulated in poly(D-L-Lactide) (PLA) polymer by spray-drying with a Buchi B290 spray-dryer; likewise, PhSeZnCl powder was also spray-dried without the excipient. The MP were characterized in terms of size distribution with an accusizer C770, encapsulation efficiency by UV-vis spectrophotometry and morphology by SEM. The MP were tested on iMEFS (WT-KO) cells incubated in standard conditions (37°C, 5% CO2, DMEM) for 5 and 24 hours and were analyzed for cytotoxicity by MTT assay and morphology by fluorescence microscopy. For this purpose the MP were labeled with FITC.

Results. The PLA MP loaded with PhSeZnCl showed spherical shape, narrow size distribution (5.34 μm), and a good content (16.9 ± 0.4%). The PhSeZnCl spray-dried powder had a comparable size distribution (5.48μm) but a more irregular morphology. The in vitro analysis showed that the formulation of PhSeZnCl in MP decreased the toxicity in iMEFS-KO cells, at 4 hours after incubation, respect to the PhSeZnCl spray-dried powder and solutions, while no differences were observed in iMEFS-WT cells. The first studies of fluorescence microscopy showed that the formulation of PLA MP are partially internalized by cells.

Conclusions. The formulation of PhSeZnCl in PLA MP seems to be potentially useful to reduce dose and time-dependent toxicity of the compound. Such formulations may have proper characteristics to be administered as a dry powder in the lungs.

LONG-CHAIN CATIONIC DERIVATIVES OF PTA (1,3,5-TRIAZA-7-PHOSPHAADAMANTANE) BASED SLN AS NEW POTENTIAL NON-VIRAL VECTORS

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Purpose. The purpose of this study was to investigate the potential of solid lipid nanoparticles (SLN) containing new positively charged detergent as nanocarriers for nucleic acids. The cationic character of SLN was obtained by adding as cationic molecules two different long-chain cationic phosphines (CP), namely hexadecyl-PTA iodide (CP16) and octadecyl-PTA iodide (CP18).

Methods. CP16 and CP18 have been prepared as iodides by treating in acetone PTA with C16H33I and C18H37I, respectively. After production, morphology and dimensions of SLN were characterized by cryo-TEM and photon correlation spectroscopy.

Then, electrophoretic mobility of complexes between CP-SLN and DNA and stability of CP-SLN/pDNA complexes towards fetal calf serum (FCS) contained nucleases were analysed. Finally, the effect of CP-SLN on cell proliferation on cultured human leukemic K562 cells and transfection activity on BHK-2 cells were investigated.

Results. N-alkyl PTA derivatives CP16 and CP18 have been prepared in high yields by reacting 1,3,5-triaza-7-phosphaadamantane (PTA) with the appropriate alkyl iodide. The use of pure tristearin for producing CP-SLN allows the obtaining of stable and homogenous dispersions, free from aggregates. The obtained CP-SLN are characterized by a positive charge on the surface and reproducible dimensions around 220 nm. These nanosystems are able to efficiently bind nucleic acid molecules and to protect DNA from the activity of serum nucleases up to 120 min. Lastly, in vitro experiments demonstrated that CP-SLN exhibit a quite pronounced antiproliferative effect on cultured human K562 erythroleukemic cells and a limited effect as transfecting adjuvant.

Conclusions. These data, and particularly the ability of CP-SLN to protect DNA from degradation, encourages further studies aimed at proposing these nanosystems as a potential approach to deliver nucleic acid to cells in living organisms.

DESIGN AND INVESTIGATION OF KETOPROFEN LYSINATE DRY POWDERS FOR INHALATION.

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Purpose. Pulmonary inflammation is an important therapeutic target in cystic fibrosis (CF) patients, aiming to limit and delay the lung damage. The purpose of this work was: to develop DPIs of ketoprofen lysinate (Klys) and to evaluate their permeation properties through an artificial lung mucus model.

Methods. Klys dry powders were manufactured by spray drying different liquid feeds, either without excipients or by adding leucine (leu) as dispersibility enhancer. DPIs were characterized in terms of drug content and technological properties (i.e. particle morphology, thermal behaviour and size distribution). Aerodynamic properties were analyzed by Andersen Cascade Impactor. For permeation studies, a mucus model was prepared, taking in account composition and viscosity of CF mucus. Drug permeation from dry powders was studied by means of Franz-type vertical diffusion cells, with or without an artificial mucus layer on the synthetic membrane.

Results. Klys dry powders were successfully produced by spray drying with high yield and optimal aerodynamic properties. Very interesting results were obtained using a 7/3 water/isopropyl alcohol mixture as liquid feed with 15% leu (yield 78%, FPF 42%). Particle size analysis showed a dramatic reduction of geometric diameter (d50) thanks to the presence of the organic co-solvent and leu into the feed. SEM analysis confirmed the reduction in particle size and the change in particle shape and morphology.
As to drug permeation, the spray-dried neat Klys powder showed a diffusion controlled release. The addition of a less polar additive such as leu had no effect on the drug permeation process, while the mucus layer slowed down Klys dissolution and permeation, acting as a physical barrier to the permeation of the drug.

Conclusions. Klys powders can be produced by spray drying with good process yield, low cohesivity and reduced geometric diameter. Leu led to micronized powders with an excellent emitted dose and satisfying fine particle fractions. The use of a synthetic mucus in the permeation studies evidenced that after the spray drying process Klys, a very polar drug, retains its ability to dissolve and permeated through an artificial mucus layer, with no influence of the excipient.

EFFECT OF IONTOPHORESIS ON TRANS-SCLERAL PERMEATION OF CYTOCHROME C

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Purpose. New therapies for posterior segment diseases involve the use of macromolecules, currently administered by repeated intravitreal injection. Iontophoretic trans-scleral delivery could be a promising non-invasive alternative. The aim of this work was to investigate the effect of iontophoresis on trans-scleral delivery of cytochrome c, a positively charged peptide (12.4 kDa) chosen as model compound.

Methods. Permeation experiments, both passive and current-assisted, were performed in Franz-type diffusion cells using porcine sclera as a barrier. The receptor compartment, filled with degassed HEPES solution (25 mM, pH 7.4), was thermostated at 37 ºC. Anodal iontophoresis was applied for two hours (permeation followed up to 5 hours) using Ag/AgCl electrodes and salt bridges. The effect of current density (1.5, 3 and 6 mA/cm2), donor solution composition and cytochrome c concentration (5, 10, 40 and 70 mg/ml) were tested. Experiments were also performed using a neutral permeant (FD-150, 1 mg/ml), alone or in combination with cytochrome c (5 or 70 mg/ml), to assess the effect of cytochrome c on electroosmotic flow.

Results. Iontophoresis enhanced cytochrome c transport across the sclera proportionally with the raise of current density. When 6 mA/cm2 were applied, the amount permeated after 2 hours increased 30 times compared to passive diffusion. Neither ionic strength of the vehicle nor the presence of neutral polymers in the donor solution affected permeation. On the contrary negatively charged polymers prevented it. Permeant concentration in the donor solution affected the extent of transport, but the enhancement was not strictly proportional to the raise of concentration. This could be due to the hindering effect (concentration dependent) of cytochrome c on electroosmotic flow, as confirmed by experiments performed with FD-150.

Conclusions. These results indicate that anodal iontophoresis could be an effective strategy to promote cytochrome c trans-scleral permeation.

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