laboratory BIOmaterials encapsulation

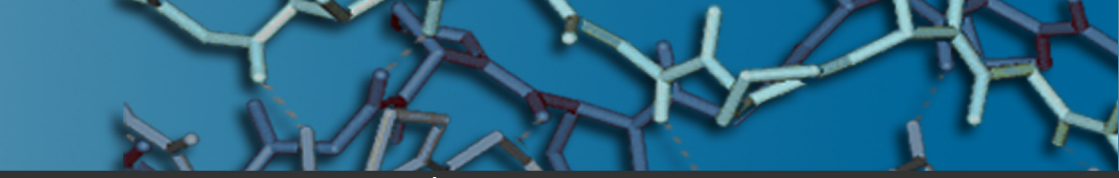
Università degli Studi di Perugia

Dipartimento di
Chimica e Tecnologia del Farmaco

BIOMATERIALS University of Perugia

CLAUDIO NASTRUZZI
Attività Scientifica e Didattica

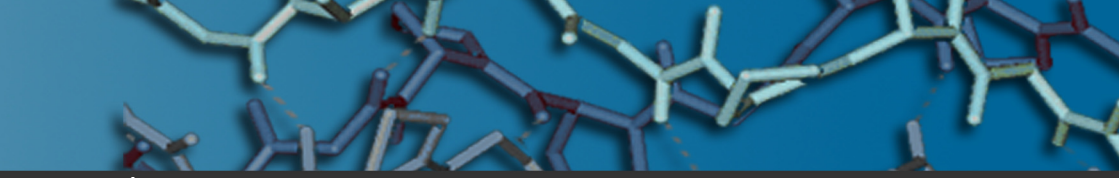
Anno Accademico 2006-2007



Percorso formativo e attività scientifica

1988-1998

Claudio Nastruzzi nato a Ferrara il 29/3/1958.
1983 - laurea in Chimica e Tecnologia Farmaceutiche presso l'Università degli Studi di Ferrara
1982-1984 - attività di ricerca, prima come laureando, poi come allievo interno, presso il Dipartimento di Scienze Farmaceutiche della Università di Ferrara. Svolge attività di ricerca nei seguenti settori:
1986-1988 - Dottorato di Ricerca in Scienze Farmaceutiche.
1988-1990 - Post-dottorato presso l'Institut für Polymere, dell'Eidgenossische Technische Hochschule (ETH) di Zurigo (Politecnico Federale Svizzero).
1990 - Responsabile di un progetto di ricerca finalizzato ETH-LVMH (LVMH è una multinazionale che comprende i marchi Dior, ROC e Givenchy).
1991-1998 - Ricercatore universitario presso il Dipartimento di Scienze Farmaceutiche,Università di Ferrara, settore disciplinare CHIM/09 Farmaceutico Tecnologico Applicativo.
Durante l'attività presso l'ateneo ferrarese il dott. Nastruzzi é stato nominato professore incaricato (A.A. 1992-1998) per l'insegnamento di Tecnologia Socio-Economia e Legislazione Farmaceutica.
Il dott. Nastruzzi é stato eletto, per due mandati, come rappresentante dei Ricercatori in Consiglio di Facoltà di Farmacia e in Giunta di Dipartimento; é stato inoltre eletto quale rappresentante dei ricercatori per il Senato Accademico Allargato per la stesura del nuovo Statuto dell' Università di Ferrara.



Attività didattica e scientifica

1990-2007

Dal 1998 - Professore Associato per il settore disciplinare Chim/09, FARMACEUTICO TECNOLOGICO APPLICATIVO, presso il Dipartimento di Chimica e Tecnologia del Farmaco dell'Università degli Studi di Perugia.
L'attività didattica del prof. Nastruzzi comprende i seguenti insegnamenti.

Tecnologia socio-economica e legislazione farmaceutiche
LAUREA SPECIALISTICA IN CHIMICA E TECNOLOGIA FARMACEUTICHE

Controlli tecnologici delle forme farmaceutiche
LAUREA TRIENNALE IN CONTROLLO DI QUALITA' NEL SETTORE INDUSTRIALE FARMACEUTICO ED ALIMENTARE

Vettori non virali per terapia genica
LAUREA SPECIALISTICA IN BIOTECNOLOGIE FARMACEUTICHE

Dal 2001 ha avviato l'attività del Laboratorio di Biomateriali e Bioincapsulazione, sviluppando varie tematiche di ricerca tra cui:
(a) Produzione e caratterizzazione di sitemi nano- e micro-particellari per il rilascio controllato di farmaci; (b) Progettazione e realizzazione di conduits per la riparazione di lesioni nervose periferiche; (c) Studio di metodiche basate su piattaforme microfluidiche su Lab-on-a-chip per la produzione di microparticelle; (d) Progettazione di formulazioni per la somministrazione orale di fitonutrienti e integratori alimentari (microparticelle gastroresistenti); (e) Sviluppo di metodiche di produzione per liposfere e SLN (Solid Lipid Nanoparticles); (f) Studio di protocolli per la immobilizzazione cellulare da utilizzare per trapianti cellulari: approcci multifunzionali per la microincapsulazione di isole pancreatiche e cellule del Sertoli; (g) Produzione e caratterizzazione di liposomi, micelle e microemulsioni per uso cosmetico; (h) Studio di processi per Dry coating su bassina e letto fluido; (i) Sviluppo di nuovi strumenti per microincapsulazione tramite approcci di virtual prototyping e tecnologie CAM/CAD/CAE.

Il prof. Nastruzzi ha al suo attivo oltre 90 pubblicazioni edite da riviste internazionali e oltre 80 comunicazioni a Congressi nazionali e internazionali.

Tecnologia socio-economica e legislazione farmaceutiche
LAUREA SPECIALISTICA IN CHIMICA E TECNOLOGIA FARMACEUTICHE

Programma.
1. Principi chimico-fisici in tecnica farmaceutica.
Soluzioni e loro proprietà, il processo di dissoluzione. Fenomeni di superficie ed interfaccia, tensione ed energia libera superficiale ed interfacciale. Sistemi dispersi. Sospensioni, caratteristiche chimico-fisiche, eccipienti, controlli, invecchiamento accelerato. Emulsioni, tipi di emulsioni, emulsionanti e tensioattivi (proprietà e classificazione), metodi di preparazione, controlli e stabilità. Soluzioni micellari e microemulsioni, diagrammi di fase, caratteristiche chimico-fisiche, applicazioni farmaceutiche.
2. Forme Farmaceutiche.
Preparati per uso orale: soluzioni, sospensioni ed emulsioni. Polveri: classificazione, mescolamento. Granulati. Capsule di gelatina molle e rigida. Compresse, tipi di compresse, eccipienti, tecnologie di produzione, controlli, compresse speciali. Rivestimento e Filmatura. Preparati per uso topico: assorbimento percutaneo, formulazioni paste, unguenti, creme, geli, lozioni, sistemi transdermici. Preparati oftalmici: colliri, pomate e inserti oftalmici. Aerosoli: tecniche di produzione, metodi di dispensazione, controlli su preparati e contenitori.
3. Rilascio Controllato di Farmaci.
Liposomi: caratteristiche generali, metodi di produzione e caratterizzazione. Microsfere e microcapsule: caratteristiche generali, metodi di produzione e caratterizzazione. Formulazioni per farmaci proteici. Profarmaci polimerici. Pompe per infusione. “Drug targeting”: coniugazione con anticorpi e molecole vettore.
4. Legislazione Farmaceutica.
Organizzazione sanitaria nazionale ed europea. Professioni sanitarie. L’esercizio della farmacia: norme nazionali e regionali. Competenze e responsabilità professionali del farmacista. Disimpegno del servizio farmaceutico. Norme sui medicinali per uso umano e veterinario. Norme riguardanti veleni e sostanze stupefacenti e psicotrope. Classificazione amministrativa dei medicinali. Ricetta medica, etichetta e foglio illustrativo. Normativa sui presidi medico-chirurgici. Il sistema brevettuale. Le procedure nazionali ed europee per l’autorizzazione all’immissione in commercio dei medicinali. Il Servizio Sanitario Nazionale: organizzazione e funzioni. Tutela dell’ambiente e la protezione dei lavoratori dell’industria farmaceutica.
5. Socioeconomia Farmaceutica.
Il sistema sanitario italiano e i sistemi degli altri Stati della U.E. ed altri stati industrializzati. Spesa sanitaria pubblica. Il mercato farmaceutico. L’industria farmaceutica: strategie di investimento. Norme di buona fabbricazione. Informazione sul farmaco. La valutazione del farmaco in base al rapporto costo/beneficio e alle dimensioni cliniche di efficacia, tollerabilità e utilità. Aspetti generali dell’analisi dei costi.

Controlli tecnologici delle forme farmaceutiche

LAUREA TRIENNALE IN CONTROLLO DI QUALITA' NEL SETTORE INDUSTRIALE FARMACEUTICO ED ALIMENTARE

Programma.

1. Principi chimico-fisici in tecnica farmaceutica.

Soluzioni e loro proprietà, il processo di dissoluzione. Fenomeni di superficie ed interfaccia, tensione ed energia libera superficiale ed interfacciale. Sistemi dispersi. Sospensioni, caratteristiche chimico-fisiche, eccipienti, controlli, invecchiamento accelerato. Emulsioni, tipi di emulsioni, emulsionanti e tensioattivi (proprietà e classificazione), metodi di preparazione, controlli e stabilità. Soluzioni micellari e microemulsioni, diagrammi di fase, caratteristiche chimico-fisiche, applicazioni farmaceutiche.

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Preparati per uso orale: soluzioni, sospensioni ed emulsioni. Polveri: classificazione, mescolamento. Granulati. Capsule di gelatina molle e rigida. Compresse, tipi di compresse, eccipienti, tecnologie di produzione, controlli, compresse speciali. Rivestimento e Filmatura. Preparati per uso topico: assorbimento percutaneo, formulazioni paste, unguenti, creme, geli, lozioni, sistemi transdermici. Preparati oftalmici: colliri, pomate e inserti oftalmici. Aerosoli: tecniche di produzione, metodi di dispensazione, controlli su preparati e contenitori.

Vettori non virali per terapia genica

LAUREA SPECIALISTICA IN BIOTECNOLOGIE FARMACEUTICHE

Programma.

1. Formulazioni non-virali per la terapia genica.

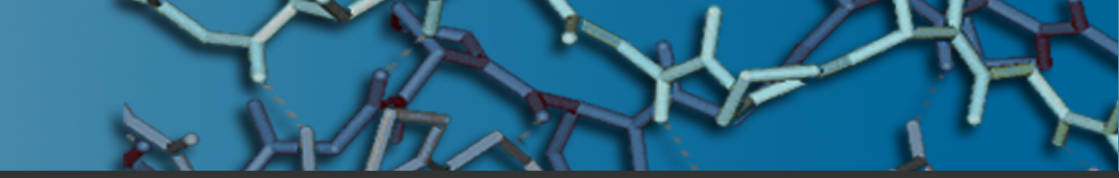
Microsfere: caratteristiche generali, metodi di produzione e caratterizzazione. Produzione di microsfere per mezzo di tecniche meccaniche: Spray-dryer, Fluidized bed, Spinning Disk, Submerged nozzle. Produzione di microsfere per mezzo di tecniche chimiche: Coacervazione, solvent evaporation, in situ cross-linking, ionic gelation.

Produzione e caratterizzazione di liposfere e solid-lipid nanoparticle (SLN).

"Drug targeting": coniugazione con anticorpi e molecole vettore.

2. Farmaci proteici.

Formulazioni per farmaci proteici. Profarmaci polimerici. Pompe per infusione.



Il Laboratorio di Biomateriali e Bioincapsulazione

Presentazione

Il Laboratorio di Biomateriali e Bioincapsulazione fa parte del Dipartimento di Chimica e Tecnologia del Farmaco dell'Università degli Studi di Perugia. Il gruppo di ricerca si occupa in particolare dello sviluppo ed applicazione di biomateriali e di formulazioni avanzate per il rilascio controllato di farmaci.

Il principale obiettivo del Laboratorio di Biomateriali e Bioincapsulazione è quello di sviluppare un concetto integrato di ricerca ed insegnamento nei settori dei biomateriali, della formulazione di farmaci e nel "drug delivery". Il gruppo è particolarmente focalizzato sulla progettazione e sviluppo di differenti formulationi per l'industria farmaceutica, biomedica, alimentare, agro-industriale e biotecnologica.

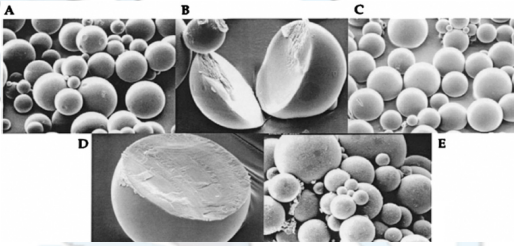
Le potenzialità di sviluppo del gruppo sono immumerevoli e comprendono: matrici inorganiche, liposomi, microparticelle, nanocapsule, microemulsioni, liposfere e film polimerici. Per lo sviluppo dei vari sistemi il Laboratorio di Biomateriali e Bioincapsulazione si può avvalere di qualificate collaborazioni esterne (vedi sezione delle collaborazioni) con laboratori che operano nei settori dell'ingegneria dei materiali, scienze mediche applicate e industria farmaceutica.

Un altro settore cruciale per il Laboratorio di Biomateriali e Bioincapsulazione è rappresentato dalle ricerche sulla incapsulazione cellulare, sugli organi bioartificiali e sui trapianti cellulari.

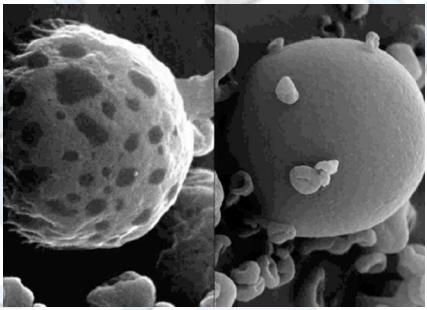
RESEARCH

NANO- AND MICRO- SYSTEMS FOR DRUG DELIVERY

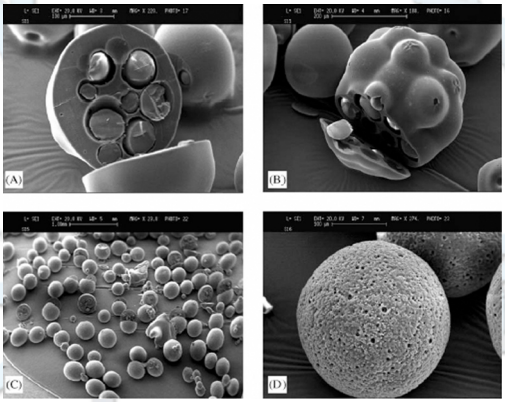
The Laboratory of Biomaterials & Bioencapsulation is involved in the design, production and characterization of controlled release systems for biological response modifiers.
Since many years we focused great part of our research interest on the production and characterization of particulate delivery systems such as liposomes, microspheres for gene therapy. In particular, new formulations, have been designed and produced for Natural and Peptide (PNA) Nucleic Acids, based on cationic polymeric sub-micron particles constituted by Eudragit RS 100 plus different cationic surfactants such as dioctadecyl-dimethyl-ammonium bromide (DDAB18) and diisobutylphenoxyethyl dimethylbenzyl ammonium chloride (DEBDA).



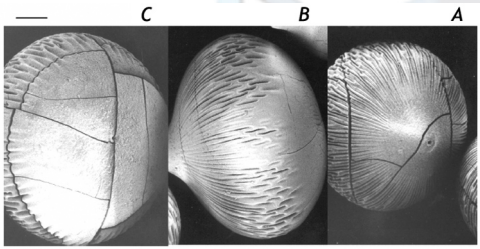
Scanning electron photographs of microparticles produced by the suspension copolymerization procedure.



SEM photographs of empty (panel A) and vitamin C (panel B) containing Eudragit RL microparticles obtained by spray drying.

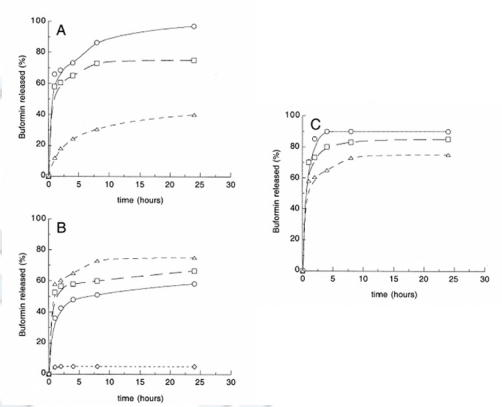


Scanning electron micrographs of Cellulose Acetate Butirrate microcapsules obtained by o/w solvent evaporation method.

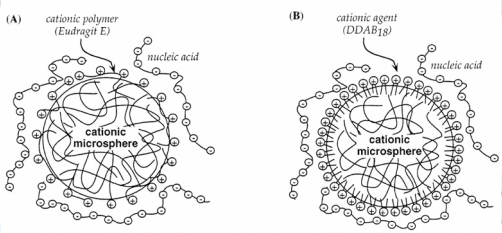


Scanning electron micrographs of the particles obtained by dripping a low-molecular weight alginate solution (8% w/v) from a distance equal to 6 cm (A), lower (B) or higher than 6 cm (C).

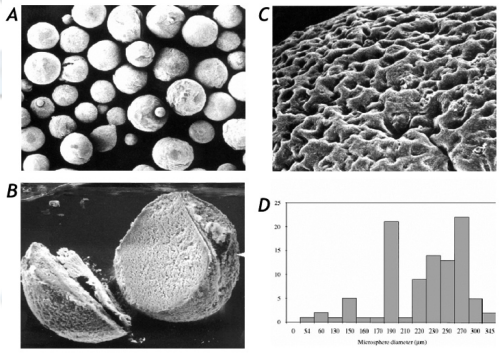
NANO- AND MICRO- SYSTEMS FOR DRUG DELIVERY



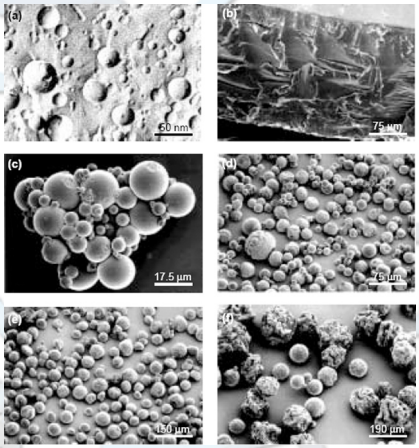
Influence of the crosslinking degree (D.C.) (panel A), MA/MM ratio (v/v) (panel B) and solvent/co-monomers ratio (v/v) (panel C) on the release behaviour of buformin (BfM) from acrylic copolymers microparticles, release experiments wereperformed using phosphate buffer (pH 7.4). Panel A: sample BfM no. 5 (circles), sample BfM no. 8 (squares), sample BfM no. 11 (triangles). Panel B: sample BfM no. 11 (diamonds), sample BfM no. 3 (circles), sample BfM no. 4 (squares), sample BfM no. 8 (triangles). Panel C: sample BfM no. 8(triangles), sample BfM no. 7 (squares), sample BfM no. 6 (circles).



Schematic representation of the hypothetical association of single or double stranded nucleic acids to positively charged microparticles. (A) Microsphere constituted of Eudragit RS and Eudragit E; (B) microsphere constituted ofEudragit RS and DDAB18.



Scanning electron micrographs of alginate microspheres (panel A and B, cross-section) obtained by emulsification method and chemical crosslinking with epichlorohydrin (panel C, surface detail). (D) Histogram of the size distribution pattern of the Ca-alginate microparticles obtained by emulsification method.

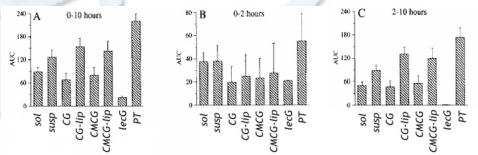


Freeze-fracture (a) and scanning electron microscopy photographs (b-f) of delivery systems for TAPP-Br. (a) Liposomes (PC:CH 4:1 mol/mol). (b) Gelatin disks. (c) Pullulan microspheres. (d) Gelatin micr spheres. (e) Dextran-treated gelatin microspheres. (f) Oxidized dextran-treated gelatin microspheres.

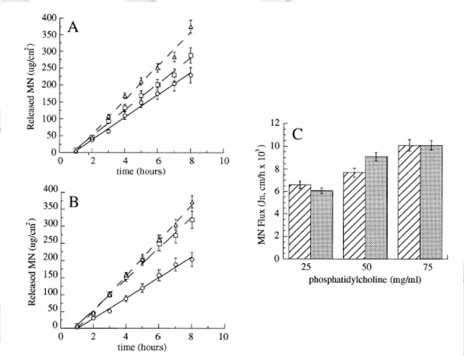
RESEARCH

LIPOSOMES, MICELLES AND MICROEMULSIONS AS DELIVERY SYSTEMS

The Laboratory of Biomaterials & Bioencapsulation has a great experience in the design and performance of specialized delivery systems (SDSs), such as liposomes, micellar solutions and microemulsions. SDSs proposed for the administration of biological response modifier are generally divided into two main categories: those defined as controlled drug-release systems and those that are target drug-release systems, depending on the kinetics of drug release and the site of action. Controlled release systems are designed both to release the drug in a predetermined and reproducible fashion and to achieve a delivery profile yielding a high blood-level of the drug over a long period of time. Targeted delivery systems are designed to improve the therapeutic index of the drug released when it selectively accumulates in specific tissues, organs or cell types.

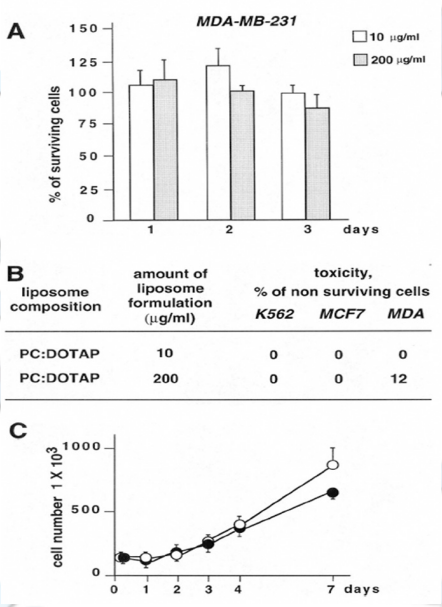


Comparative effect of different formulations on the in vivo activity of MN. Histograms represent the mean values (n=6) +/-SD of area under the curve (AUC) corresponding to the indicated length of time after product application. Sol, aqueus solution; susp, liposome suspension; CG, Carbomer based gel; CG-lip, liposome suspension incorporated in Carbomer based gel; CMCG, carboxymethyl cellulose based gekl; CMCG-lip, liposome suspension incorporated in carboxymethyl cellulose based gel; lecG; lecithin gel; PT, pretreatment.



In vitro release kinetics of methyl nicotinate from liposome suspensions (A) or in liposome suspensions viscosized by Carbomer (B). PC concentrations were 25 (), 50 () or 75 () mg:ml. (C) PC effect on Jn values for MN incorporated into aqueous liposomes (square with right diagonal lines) or into viscosized liposomes (square with criss-cross lines).

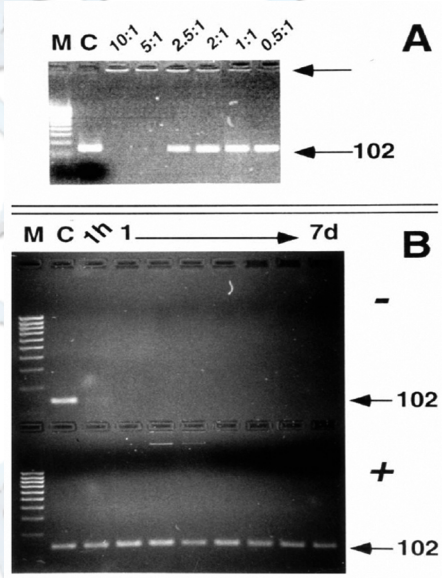
LIPOSOMES, MICELLES AND MICROEMULSIONS AS DELIVERY SYSTEMS



B

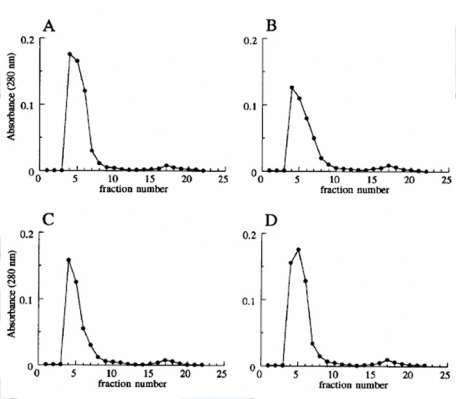
liposome composition	amount of liposome formulation (μg/ml)	toxicity, % of non surviving cells K562	MCF7	MDA
PC:DOTAP	10	0	0	0
PC:DOTAP	200	0	0	12

Effect of PC:DOTAP cationic liposomes on cell survival of in vitro cultured MDA-MB-231 breast cancer cells. In panel A the data obtained by counting the viable cells (up to 3 days of cell culture) with a colorimetric assay based on MTT (thiazolyl blue) are reported. The viability of untreated controls has been set as 100%. Results are expressed as the percentage of surviving cells and are the mean SEM of at least four independent experiments. The differences were found to be not significant with P<0.01 (from 1 to 3 days the P-values were 0.92, 0.73 and 0.057, respectively). The tested concentration 10 mg:ml corresponds to the lowest concentration at which the DNA:liposome complex is formed. The toxicity of PC:DOTAP cationic liposomes in K562 erythroleukemic cells, MCF7 and MDA MB-231 breast cancer cells, after three days of treatment, is reported in panel B. Panel C: the data on cell proliferation were determined by counting, with a cell counter, the cells that were treated with 200 mg:ml of PC:DOTAP or that remained untreated (). This assay was done in duplicate and performed three times.

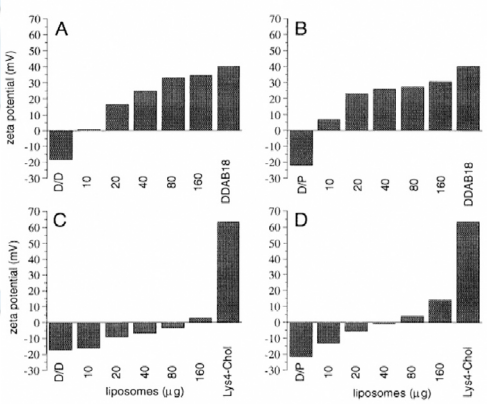


Panel A. DNA-liposome association assay. Samples containing 125 ng of DNA-102 and liposomes in various liposome-to-DNA ratios (w:w) were incubated at room temperature for 30 min and loaded on agarose gel. The associated DNA is indicated by the arrow at the top of the gel; the non-associated DNA is arrowed as 102. Mmolecular weight marker (100 bp DNA ladder); Cuncomplexed PCR-102. Panel B. Protective effect of liposomes on serum nuclease-mediated degradation of DNA-102. DNA-102 was incubated in the presence of 10% FBS in water at 37°C for 0 (C), 1 h and 1, 2, 3, 4, 5, 6, 7 days, in the presence or in the absence of liposomes. The amount of intact DNA-102 was determined by densitometric measurements of the fluorescence signals in agarose gel after ethidium bromide staining. pBLCAT8ERCAT1 [5] recombinant plasmid was used as template for DNA-102. After amplification DNAs were purified by ultrafiltration procedure with Microcon-30 system (Amicon, Beverly, USA) as previously described [14]. Mmolecular weight marker (100 bp DNA ladder).

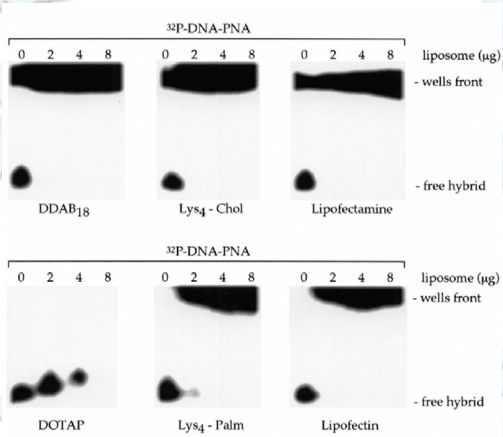
LIPOSOMES, MICELLES AND MICROEMULSIONS AS DELIVERY SYSTEMS



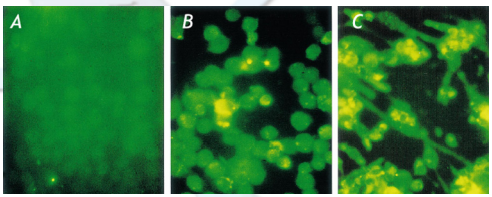
Elution profiles of liposome-associated CPT from gel permeation chromatography on Sepharose 4B column (1.3 cm internal diameter; 28 cm length; flow rate: 420 ml/min; 8.4 ml/fraction). The liposome-associated CPT were obtained by the two step method. A Egg-PC liposomes; B: Egg-PC:CH liposomes; C: Egg-PC:CH:DDAB18 liposomes; D: Egg-PC:CH:DPC liposomes.



Zeta potential of nucleic acid / cationic liposomes complexes. Panels A and C: DNA/DNA hybrids complexed to lipo-DDAB and 18 lipo-Lys-Chol, respectively. Panels B and D: DNA/PNA hybrids complexed to lipo-DDAB and lipo-Lys-Chol, respectively.



Comparison of the complexation efficiencies of P-DNA-PNA molecules to our lipo-DDAB (DDAB), lipo-DOTAP (DOTAP), 18 lipo-Lys-Chol (Lys-chol) and lipo-Lys-Palm (Lys-palm), or to the well known commercially available lipoformulations, lipofectin and lipofectamine.

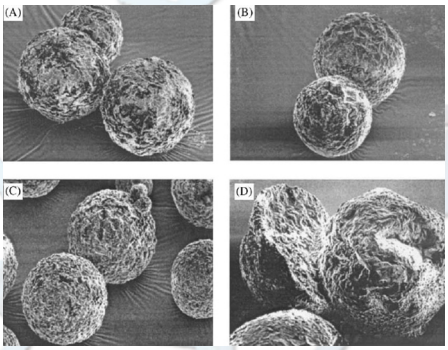


Delivery of DNA-PNA molecules into in vitro cultured J774 cells. Cells were incubated in the presence of fluorescein labeled DNA-PNA molecules (A) or in the presence of fluorescein-labeled DNA-PNA molecules complexed to lipo-DDAB 18 (B) or lipo-DOTAP (C). After 20 h of incubation, cells were fixed and analyzed by a fluorescence microscope.

LIPOSPHERES

Lipidic microspheres, lipospheres (LS) as new type of fat-based encapsulation systems developed for drug delivery of bioactive compounds (especially lipophilic compounds). LS combine the advantages of polymeric nanoparticles, fat emulsions and liposomes avoiding some of their disadvantages, such as cytotoxic effects after phagocytosis, toxic effects of organic residues after the production of polymers, lack of large industrial scale production.Lipid microspheres, often called lipospheres (LS), have been proposed as new type of fat-based encapsulation system for drug delivery of bioactive compounds (especially lipophilic compounds). LS consist of solid microparticles with a mean diameter usually comprised between 0.2 and 500 mm, composed of a solid hydrophobic fat matrix, where the bioactive compound(s) is dissolved or dispersed. LS have some advantages such as (a) good physical stability, (b) low cost of ingredients, (c) ease of preparation and scaleup and (d) high entrapment yields for hydrophobic drugs. Due to their large particle size range, LS can be administered by different routes,such as oral,sub cutaneous, intramuscular, topic or used for cells encapsulation being thus proposed for the treatment of a number of diseases. For instance, the in vivo distribution of LS demonstrated a high affinity to vascular wells, to inflamed tissues and to granulocytes. LS have been used for the controlled delivery of various types of drugs, including vasodilator and antiplatelet drugs, anti-inflammatory compounds, antibiotics and anti-cancer agents.

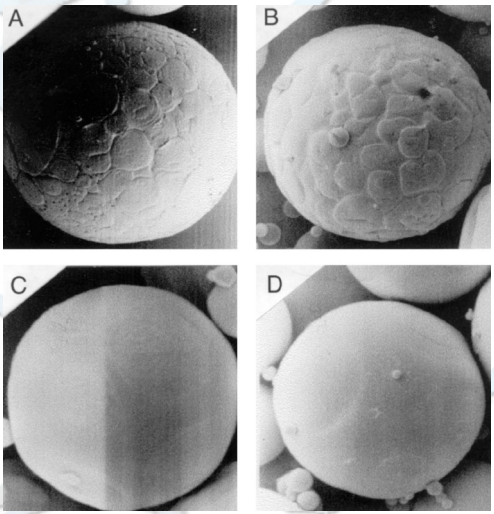
They have also been used successfully as carriers of vaccines and adjuvants. In the Laboratory of Biomaterials we can performe studies aimed to (a) the production and characterisation of LS formed by emulsion-melt dispersion technique, by solvent evaporation method, and by w/o/w double emulsion method, (b) the influence of preparation parameters on LS morphology, (c) the encapsulation efficiency and the release characteristics of two lipophilic model drugs, such as retinyl acetate and progesterone, and one hydrophilic drug, sodium cromoglycate (SCG), from the prepared LS. To obtain a biocompatible formulation suitable for human administration, triglycerides and monoglycerides have been chosen as LS' biomaterials, in reason of their high biocompatibility, high physico-chemical stability and drug delivery release.



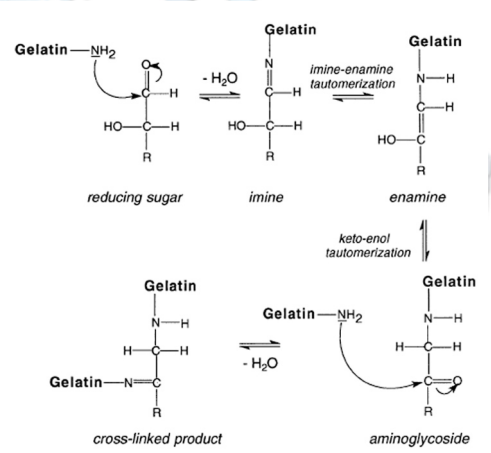
SEM photographs showing the effect of lipid composition on the morphology of LS. LS were prepared with tristearin:glyceryl monostearate 2:1 (w/w) (#25) (A),tristear in:cetyl alcohol 2:1 (w/w) (#26) (B), tripalmitin:glyceryl monostearate 2:1 (w/w) (#24) (C),and tripalmitin:cetyl alcohol 2:1 (w/w) (#23) (D).

GELATIN DEVICES

Since many years we have studied the design, production and characterization of gelatin microspheres and disks as drug delivery system. Among natural polymers (preferred for their low toxicity and biocompatibility), gelatin represents a good raw material since it easily forms films and particles. In addition, gelatin is available in a pyrogen-free form and it can be used for the production of delivery systems requiring bioadhesion to mucosal tissues. In our laboratory we have developed advanced procedures for thermal hardening treatments or 'natural' cross-linking of gelatin microparticles by sugar or sugar-derived compounds. Recently we have studied the ability of native and oxidized mono- and di-saccharides to induce the cross-linking of gelatin. To this end, gelatin discs and gelatin microspheres were produced and their dissolution kinetics at 37°C were examined. In order to find evidence of sugar-mediated cross-linking, DSC and FTIR experiments were performed. The obtained results indicated that both native and oxidized sugars resulted to different extents, in the formation of a cross-linked gelatin network able to reduce the dissolution of gelatin.
These results suggest that oxidized mono- and disaccharides could be an interesting method by which to cross-link gelatin thereby reducing the risk of toxic side e¤ects arising from the use of synthetic cross-linkers.



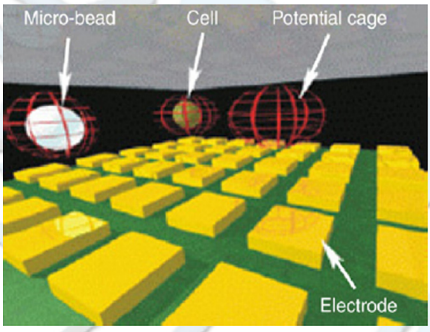
Scanning electron micrographs of gelatin microspheres. Panel A: untreated GMs; Panel B: GMs treated with native glucose; Panel C: GMs treated with native fructose; Panel D: GMs treated with native sucrose.



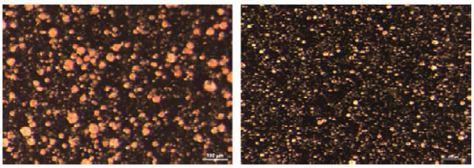
Schematic representation of the reaction mechanism of sugar-mediated gelatin cross-linking.

LAB-ON-A-CHIP

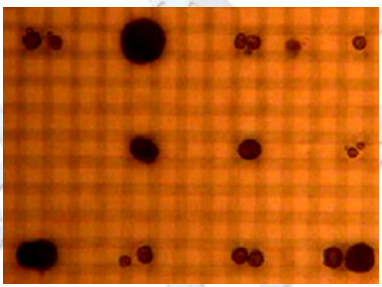
Prodution and characterization of nano- and microparticellar "system for biopharmaceutical applications on a Lab-on-a-chip based microelectronic technology". Production and characterization of polymeric microspheres. The project is devoted to the selection of the particles that optimally fit to "Lab-on-a-chip" biopharmaceutical applications. Polymeric microspheres will be produced and characterised by different experimental approaches including solvent evaporation, spray drying and thermal gelation. The development of advanced analytical and bioseparation methodologies based on micro- or macroarrays and biosensors is one of the postgenomics strategic objectives, but it also has an impact on strategic application fields, such as predictive oncology, diagnostics in the biomedical field, and drug research. We are presently investigating the performance of a Lab-on-a-chip device, called DEParray™Chip, equipped with 102,400 arrayed electrodes. This Lab-on-a-chip is able to generate more than 10,000 independent dielectrophoretic (DEP) cages, where cells and microparticles can be immobilized. Inside the chip, the movement of the cages can promote the interaction between cells and microparticles, with successive separation. Among the different microparticulate systems that can potentially fit to Lab-on-a-chip, we choose to study the applicability of cationic lipidic microspheres. These microparticles are constituted of a mixture of neutral lipids and positively charged lipids with a mean diameter that can be adjusted easily between 0.2 and 500 µm



Schematic representation of DEP cages in DEParray™Chip.



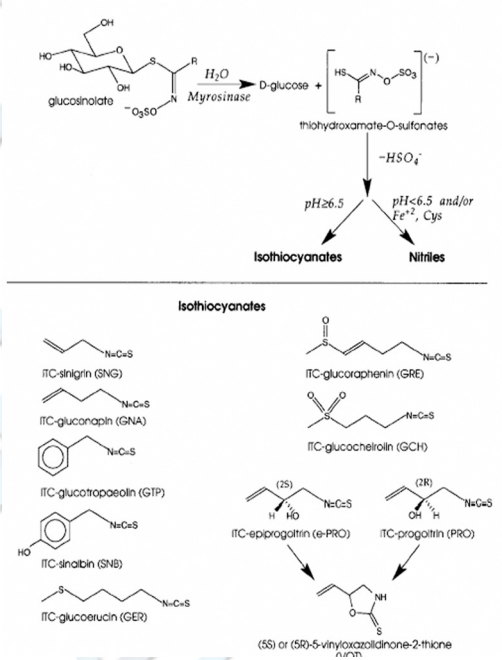
Stereomicrograph of CLS produced using different amounts of DDAB18: (A) 10% (#TCLS1) and (B) 20% (#TCLS6).



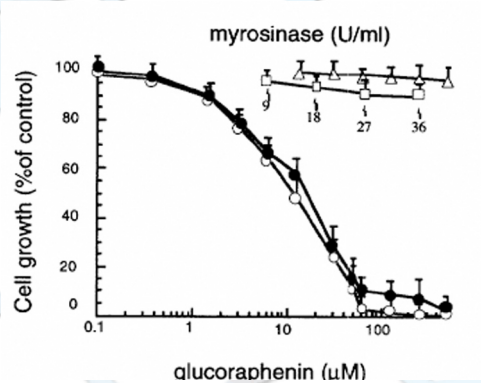
Distribution of #TCLS5 lipospheres on DEP cages, pattern cage 1gap3, generated in DEParray™Chip.

PHYTONUTRIENTS

Design, production and characterization of microparticles made of enteric resistant polymers to prevent phytonutrients release to the stomach and to the upper small intestine. After oral administration, particles can indeed protect drug during the passage through the stomach, affording site specificity and gradual release.



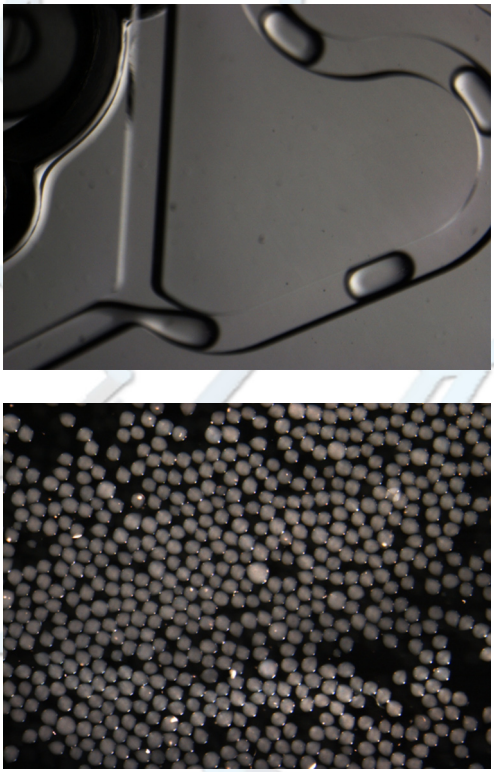
Scheme of myrosinase catalyzed hydrolysis and chemical structure of glucosinolates



Effect of myrosinase(O), GRE (D) and GRE-ITC on in vitro clutured human erythroleukemic K562 cells in the concentration range comprised between 0.1 and 500 mM. Experiments were conducted either following in the situ (O) or pre-mix methods (●). Arrows indicate the myrosinase concentration espressed in U/mL. Data represent the average of four independent determinations +/- SD.

MICROFLUIDIC

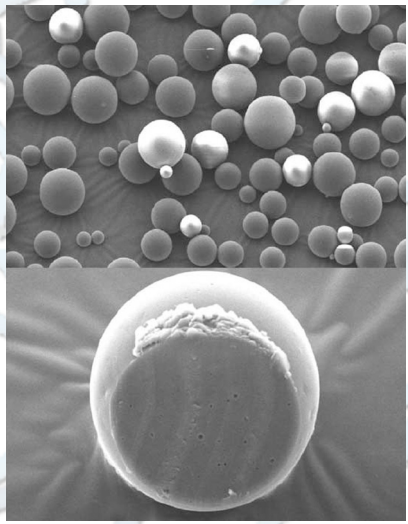
We have recently developped new method for manufacturing shape-controlled calcium alginate gel microparticles in a microfluidic device. Both manufacturing shape-controlled microparticles and synthesizing hydrogel microparticles could be performed simultaneously in the microfluidic device. The novel microfluidic device comprised of two individual flow-focusing channels and a synthesizing channel was successfully applied as a continuous microfluidic reactor to synthesize gel microparticles with size and shape control. By passive control based on the microchannel geometric confinement and liquid-phase flow rates, we succeeded in producing monodisperse sodium alginate microparticles with diverse shapes (such as plugs, disks, microspheres, rods, and threads) in the flow-focusing channels of the microfluidic device. The shape and size of the sodium alginate microparticles could be tuned by adjusting the flow rates of the various streams. Further stages of the chemical reaction could be initiated by mixing sodium alginate microparticles and calcium chloride (CaCl2) solution in the synthesizing channel. The shapes of the sodium alginate microparticles could be permanently preserved by the synthesis of calcium alginate gel microparticles. The preparation conditions of size- and shape controlled calcium alginate microparticles and influence factors were studied.



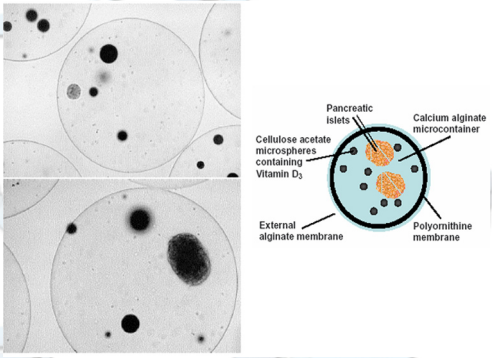
Optical stereo photomicrograph of a thinxxs snake mixer microfluidic chip (upper panel), and of agarose based microcapsules obtained by a microfluidic technology.

MULTIFUNCTIONAL MICROCAPSULES FOR ISLET ENTRAPTMENT

Great advances in cell transplantation have been made, including the recent, remarkable success in pancreatic islet transplantation for the treatment of type 1 diabetes mellitus. Unfortunately, the transplanted cells are very susceptible to oxidative stress that cause severe damage to either allo- or xenogeneic islets upon graft in diabetic patients. Consequently, the transplanted islet functional life span is significantly shortened. The aim of this study was to examine the possible effects of antioxidants on in vitro cultured adult rat islets, and to evaluate the effects of a prolonged-release formulation, in form of cellulose acetate (CA) microspheres, on Vitamin D3 activity. Isolated rat islets, both free and entrapped in microspheres were treated with Vitamin D3. The effects of the vitamin were studied at 3, 6 and 9 days of in vitro cell culture. According to insulin secretory patterns, treatment with Vitamin D3 of both free and CA entrapped microspheres, increased the insulin output as compared to untreated controls. Such positive effects were confirmed under islet static incubation with glucose at day 6. These results suggest that pancreatic islets can be advantageously treated with anti-oxidising vitamins before implantation, and speculatively, with the help of special delivery systems, throughout the islet cell life span, in the post-transplant time period.



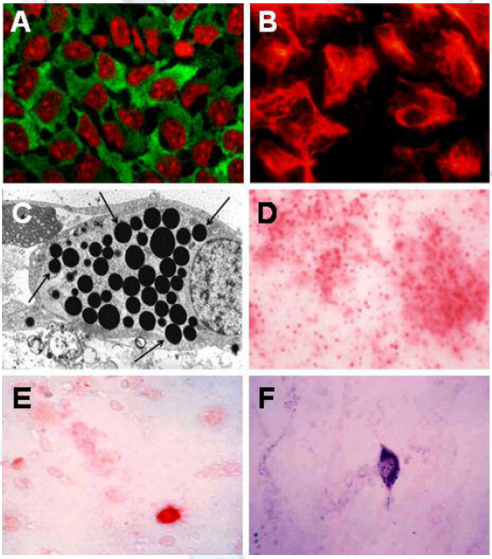
Scanning electron microphotographs of empty (upper panel) or Vitamin D3 containing (lower panel) CA microspheres.



Schematic representation of multifunctional microcapsules for islet encapsulation. Optical microphotographs of multifunctional alginate microcapsules containing both RPI and Vitamin D3 containing CA.

ENCAPSULATION OF SERTOLI CELLS

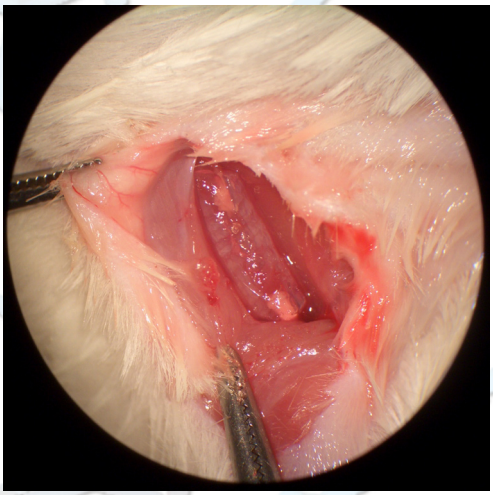
SERTOLI'S CELLS (SC) have been considered for many years to have a simple mechanical architectural function. More recently, these cells, have been re-evaluated with respect to their activity and functions. SC have been recognised to have important functions at different levels, including pathophysiological functions such as the syntesis of androgen binding protein (ABP), the paracrine action on peritubular and Leydig cells. In addition, SC provide an appropriate microenvironment for the development of germ cells. Moreover, recent reports have shown that this interesting cells may provide nutrients, immunomodulatory and trophic factors that are able to ameliorate survival and development of different cell types and improve functional competence both, in vitro and in vivo in different cells. Infact many papers have described the ability of SC to protect transplanted tissue allografts (mainly pancreatic B-cells) by formation of a testis-like immunoprivileged environment, with no need for prolonged immunosuppression therapy regimens.A method for microencapsulation of isolated neonatal porcine Sertoli cells is described. Using conventional alginate-poli-L-ornitina encapsulation procedure, that has been used in our laboratory for almost two decades to embody pancreatic islets, we observed significant loss of sertoli cells viability, possibly due to excessive Ca+2 ion exposure. Replacing calcium with barium, or shortening the incubation period in the presence of Ca ions, we have obtained barium- or calcium-alginate gel micro beads that did not alter morphology and viability of the enveloped Sertoli cells. The procedure may inaugurate a novel approach to help enhance immunological acceptance and implement viability of the cell grafts.



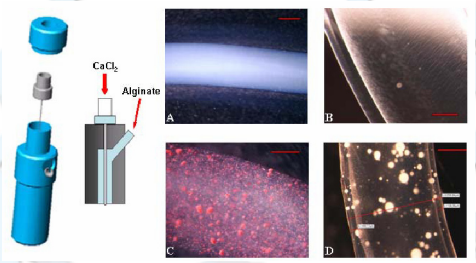
Morphological characterization of SC preparation by optical (A, D, E, F), fluorescence (B) and electron (C) microscopy. A: SC after immuno taining with specific Ab for the Mullerian inhibiting substance (MIS). B: SC after immunostai ing with anti-vimentin Ab. C: transmission elecron microscopy (TEM) showing the typical lipid bodies (see arrows) present in the SC cytoplasm. D: SC after positive staining with Sudan III. Within the non-SC cell populations, eliminated during the purification of SC, peritubular cells constituted a minimal fraction out of the isolated cell mass (E), Leydig cells were d tected at negligible concentrations (F). Bar corresponds to 10, 5, 0.2, 5, 10, 5 µm in panel A, B, C, D, E and F, respectively.

CONDUITS FOR NERVE REPAIR

Development and optimization of different bioencapsulation methods in view of their selection and transfer to cell transplantation protocols with special regard to Sertoli cells transplantation in conduits for nerve repair. Additionally, specific formulations for the controlled release of biomodulators will be developed in an attempt to possibly provide the grafts with long-term stability in terms of both, capsular stability and cell viability.
Further aims are to explore the possibilities offered by new materials in cell encapsulation and to identify new processes allowing the use of these materials under mild and biocompatible conditions.



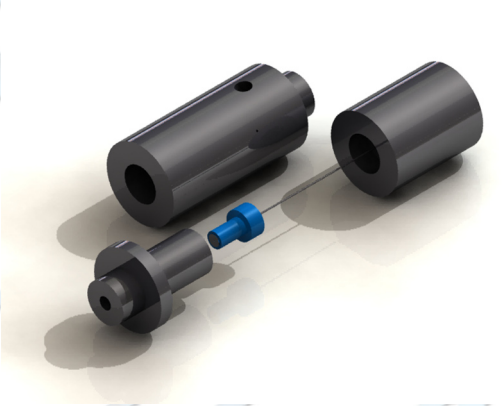
Optical stereo photomicrograph of a polimeric nerve conduit implanted in mice for the repair of the sciatic nerve

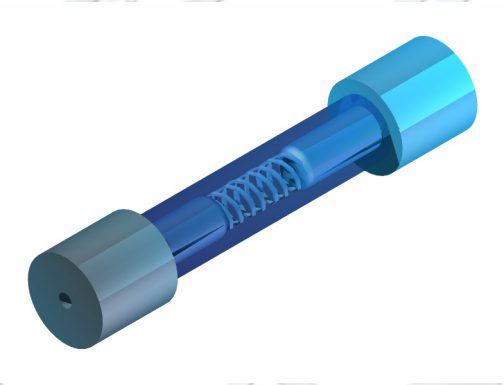


Left part: Schematic rappresentation of the LB-EXT/1 (Laboratory of Biomaterials-Extruder/1). Right part: Microphotography (A) of microtube based on alginate IE 1105 3 % (p/p) product with LB-EXT/1, (the bar length is 1250 µm); microphotography (B) of microtube based on alginate IE-1105 3 % (p/p) product with LB-EXT/1, (the bar length is 500 µm); microphotography (C) of microcylinders based on alginate IE-1105 3% (p/p) product with LB-EXT/1, with microspheres incorporated based on Eudragit RS100 (the bar length is 500 µm); Microphotography (D) of microtubes based on alginate Protanal LF 20/40 3 % (p/p) product with LBEXT/ 1, with microspheres incorporated based on Cellulose Acetate (the stick length is 1250 µm) (the bar length is 1250 µm).

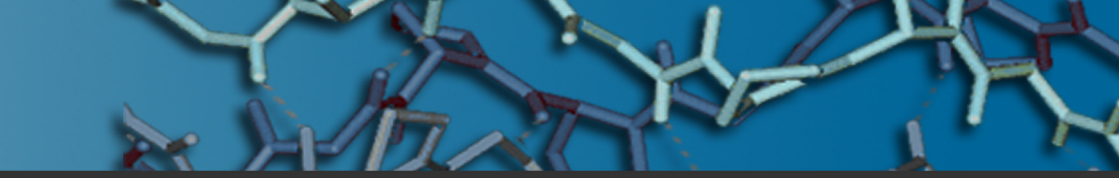
VIRTUAL PROTOTYPING

Virtual prototyping is the process of using a virtual prototype, in lieu of a physical prototype, for test and evaluation of specific characteristics of a product design.We offer design of virtual 3d prototypes for static and dynamics models. We can produce three-dimensional objects very quickly, without companies having to invest in expensive tooling techniques during the product development stage. These are computer-based design and manufacture processes, which are capable of producing high-precision parts from computer-generated data, rapidly and automatically.





3-dimensional sketches of CAD/CAM/CAE generated ptototypes. Thermostated tank, extusor, static mixer.



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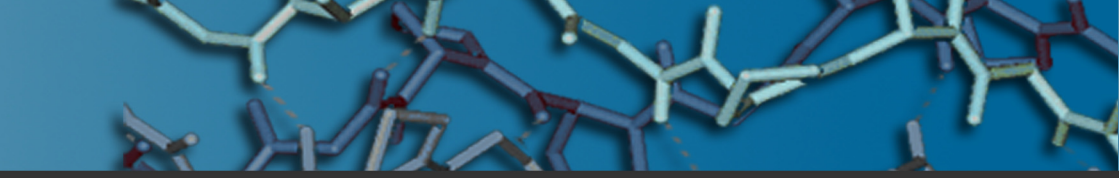

Collaborazioni

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Dept. Food Technology and Biochemistry
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University of Belgrade
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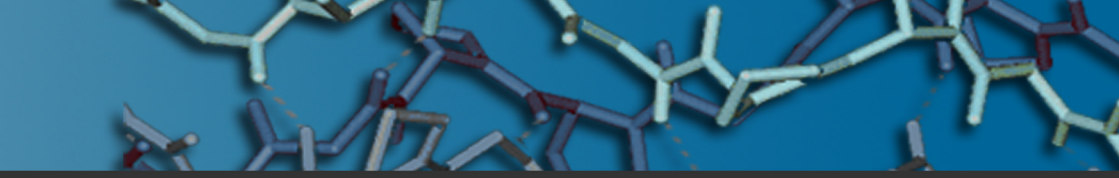


Partecipazione ai progetti europei COST 840 & 865

Il Laboratorio di Biomateriali e Bioincapsulazione partecipa da anni attivamente alle azioni COST 840 e 865.
Il prof. Nastruzzi é attualmente rappresentante italiano nel management commitee dell'azione COST 865 : Bioencapsulation multiscale interaction analysis.

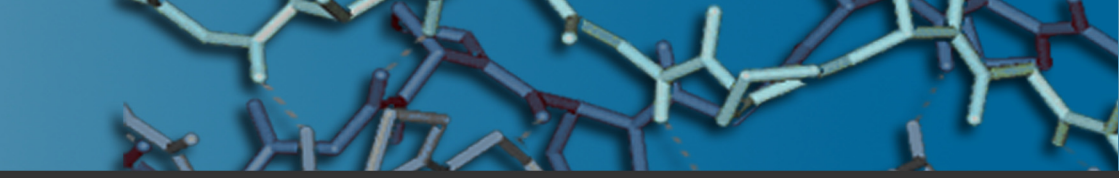
Cost actions are transnational programs (35 countries, mainly European) to allow Coordination in Sciences and Technologies of research on a specific topic. COST finances workshop organisation and participation (travel and daily allowance, expert meetings, publications and Short term scientific meetings.

The main objective of the COST865 is to improve the knowledge on bioencapsulation in developing reliable, economical and safe industrial encapsulation processes and applications by connecting researchers and industrials in Bioencapsulation and promoting exchanges and colaborations.

The action is divided in 4 working groups, developping collaborative works at different level:

* WG I : Molecular Level (Materials, Characteristics, Intercations, compatibility ...)
* WG II : Microcapsules level (Physical properties, mass transfer, Biological interac tions ...)
* WG III : Technological Level (Processes, cost, environment ...)
* WG IV : Application Level (Applications, GMP ...)

COST865 Summer School
Summer 2008
Athena, Greece



PUBLICATIONS

Scientific articles

1: Drug Deliv. 2007 Jan-Feb;14(1):1-8.
Liposomes and micellar dispersions for delivery of benzoheterocyclic derivatives of distamycina.
Cortesi R, Esposito E, Cuccu I, Romagnoli R, Menegatti E, Zaid AN, Nastruzzi C.
In this article we describe the production and characterization of specialized delivery systems for some distamycin derivatives (DD), namely liposomes and micellar dispersions. All the formulations were designed to increase the solubility of DD in an aqueous environment and to reduce the possible toxicity problems related to the administration of these drugs. For instance, liposomes were prepared by reverse phase evaporation technique followed by extrusion through polycarbonate filters, then characterized in terms of dimensions, morphology, and encapsulation efficacy. The analysis of their in vitro antiproliferative activity on cultured human and mouse leukemic cells demonstrated that liposomes and micellar dispersions containing DD exert quite different effects. These effects were compared with those shown by the free drug depending on type of drug and also cell line used.

2: Cell Transplant. 2006;15(1):55-65.
Effects of simulated microgravity on the morphology and function of neonatal porcine cell clusters cultured with and without Sertoli cells
Luca G, Calvitti M, Nastruzzi C, Macchiarulo G, Becchetti E, Neri LM, Capitani S, Basta G, Brunetti P, Calafiore R, Cameron DF.
Human islet allografts are well known to induce full and sustained remission of hyperglycemia, with complete normalization of key metabolic parameters. Nevertheless, acquiring human islets, even from cadaveric human donor pancreases, remains a significant impediment to successful transplantation therapy for diabetes. To overcome this difficulty, neonatal porcine cell clusters (NPCCs) have been considered for human islet substitutes because they are easily obtained by collagenase digestion of the neonatal piglet pancreas. Currently, the major hurdle in using NPCCs for xenograft is the delay (time lag) in achieving the posttransplant normalization of blood glucose levels in animal diabetic recipients. The present work is the first attempt to evaluate whether incubation of NPCCs in simulated microgravity, in the presence or absence of Sertoli cells (SC), may reduce the maturation time lag of beta-cells by differentiation acceleration in vitro, thereby expediting production, viability, and acquisition of functional competence of pretransplantation beta-cell-enriched islets. Following a 3-day incubation period, NPCCs maintained in conventional culture, NPCCs incubated in simulated microgravity in the HARV biochamber, and NPCCs plus co-incubated SC in simulated microgravity were examined for viability, morphology, and insulin secretion. Results show that NPCCs grown alone in the HARV biochamber are superior in quality, both in terms of viability and functional competence, when compared to other culture pretreatment protocols. This finding strongly suggests that NPCC pretreatment in simulated microgravity may enhance the transplantation success of NPCCs in the diabetic recipient.

Scientific articles

3: Int J Oncol. 2005 Dec;27(6):1559-66.
Dielectrophoresis-based 'Lab-on-a-chip' devices for programmable binding of microspheres to target cells.
Borgatti M, Altomare L, Abonnec M, Fabbri E, Manaresi N, Medoro G, Romani A, Tartagni M, Nastruzzi C, Di Croce S, Tosi A, Mancini I, Guerrieri R, Gambari R.
There is a general agreement on the fact that the Laboratory on chip (Lab-on-a-chip) technology will enable laboratory testing to move from laboratories employing complex equipments into non-laboratory settings. In this respect, dielectrophoresis (DEP) is a very valuable approach to design and produce Lab-on-a-chip devices able to manipulate microparticles and cells. In this study, we report the application of DEP-based devices for facilitating programmable interactions between microspheres and target tumor cells. We used two Lab-on-a-chip devices, one (the SmartSlide) carrying 193 parallel electrodes and generating up to 50 cylinder-shaped DEP cages, the other (the DEP array) carrying 102,400 arrayed electrodes and generating more than 10,000 spherical DEP cages. We determined whether these devices can be used to levitate and move microspheres and cells in order to obtain a forced interaction between microspheres and target cells. The first major point of this manuscript is that the DEP-based SmartSlide can be used for transfection experiments in which microspheres and target cells are forced to share the same DEP cage, leading to efficient binding of the microspheres to target cells. The data obtained using the DEP array show that this system allows the sequential, software-controlled binding of individually and independently moved single microspheres to a single target tumor cell. To our knowledge, this is the first report on the possible use of a DEP-based Lab-on-a-chip device for guided multiple binding of singularly moved microspheres to a single tumor cell. This approach can be of interest in the field of drug discovery, delivery and diagnosis.

Scientific articles

4: J Pharm Pharmacol. 2005 Sep;57(9):1169-76.
Effect of charge and lipid concentration on in-vivo percutaneous absorption of methyl nicotinate from liposomal vesicles.
Puglia C, Esposito E, Menegatti E, Nastruzzi C, Rizza L, Cortesi R, Bonina F.
We have investigated the influence of charge and lipid concentration on the in-vivo percutaneous absorption of a model compound, methyl nicotinate (MN), from liposomal vesicles. MN-loaded liposomes were produced by the reverse-phase evaporation method (REV) using different concentrations of phosphatidyl choline (PC), in association with surfactants such as dioctadecyl dimethyl ammonium bromide (DDAB18) and dicetyl phosphate (DCP), which impart a positive or negative charge to the systems, respectively. The liposomal suspensions were then processed to hydrogels and used to study in-vivo the MN permeation profile. MN was chosen as the model compound since it was capable of causing cutaneous erythema, the intensity and duration of which was proportional to the amount entering the living epidermis over time. The extent of the erythema was monitored by reflectance spectrophotometry, a non-invasive technique. In-vivo findings showed an interesting MN delayed release, which was proportional to the amount of phospholipids in each liposomal formulation. Furthermore, it could be noted that the erythematous effect was more prolonged when MN was delivered from neutral or negatively-charged liposomal forms.

5: Biomaterials. 2005 Jul;26(20):4337-47.
Cellulose acetate butyrate microcapsules containing dextran ion-exchange resins as self-propelled drug release system.
Fundueanu G, Constantin M, Esposito E, Cortesi R, Nastruzzi C, Menegatti E.
Sulfopropylated dextran microspheres (SP-Ms), (Dm = 80 microm) loaded with a water soluble drug (Tetracycline HCl), were included in cellulose acetate butyrate (CAB) microcapsules. Spherical CAB microcapsules were obtained by oil in water (o/w) solvent evaporation method in the presence of an inert solvent as cyclohexane (CyH) or n-hexane (N-Hex), and different excipients (Phospholipon, Tween, Span, Eudragit RS 100). Chloroform was found to be the best solvent for the preparation of the microcapsules. Also, the sphericity as well as the porosity of the microcapsules was controlled by the presence of an inert solvent. The final concentration of the drug in CAB microparticles was up to 25% (w/w). The key factors for the successful preparation were also the viscosity of the polymer, while the wettability of the resulted microcapsules, the temperature of the preparation, and the porosity have modulated the release of the drug. The higher is the amount of encapsulated microspheres the thinner is the CAB wall between the compartments created by their incorporation. When these microspheres come in contact with the release medium, the pressure created by their swelling breaks the polymer film and the drug starts to be released. The more drug is released in phosphate buffer the higher is the swelling degree of the encapsulated ion exchange resins and the force created by their supplementary swelling will break the more resistants walls. In this way a self-propelled drug release is achieved, until almost all drug was eliberated.

Scientific articles

6: Cell Transplant. 2005;14(5):249-61.
Accelerated functional maturation of isolated neonatal porcine cell clusters: in vitro and in vivo results in NOD mice.
Luca G, Nastruzzi C, Calvitti M, Becchetti E, Baroni T, Neri LM, Capitani S, Basta G, Brunetti P, Calafiore R.
Neonatal porcine cell clusters (NPCCs) might replace human for transplant in patients with type 1 diabetes mellitus (T1DM). However, these islets are not immediately functional, due to their incomplete maturation/ differentiation. We then have addressed: 1) to assess whether in vitro coculture of islets with homologous Sertoli cells (SC) would shorten NPCCs' functional time lag, by accelerating the beta-cell biological maturation/ differentiation; 2) to evaluate metabolic outcome of the SC preincubated, and microencapsulated NPCCs, upon graft into spontaneously diabetic NOD mice. The islets, isolated from < 3 day piglets, were examined in terms of morphology/viability/function and final yield. SC effects on the islet maturation pathways, both in vitro and in vivo, upon microencapsulation in alginate/poly-L-ornithine, and intraperitoneal graft into spontaneously diabetic NOD mice were determined. Double fluorescence immunolabeling showed increase in beta-cell mass for SC+ neonatal porcine islets versus islets alone. In vitro insulin release in response to glucose, as well as mRNA insulin expression, were significantly higher for SC+ neonatal porcine islets compared with control, thereby confirming SC-induced increase in viable and functional beta-cell mass. Graft of microencapsulated SC+ neonatal porcine islets versus encapsulated islets alone resulted in significantly longer remission of hyperglycemia in NOD mice. We have preliminarily shown that the in vitro NPCCs' maturation time lag can dramatically be curtailed by coincubating these islets with SC. Graft of microencapsulated neonatal porcine islets, precultured in Sertoli cells, has been proven successful in correcting hyperglycemia in stringent animal model of spontaneous diabetes.

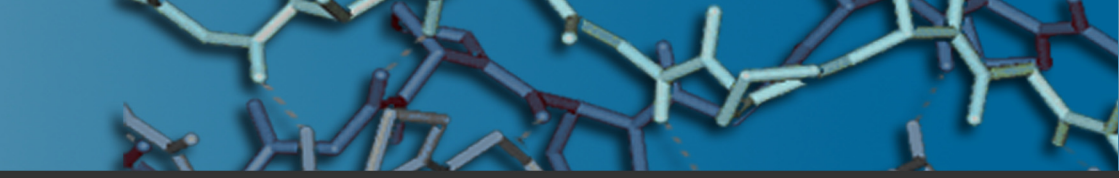
Scientific articles

7: Transpl Immunol. 2004 Dec;13(4):289-96.
Optimized parameters for microencapsulation of pancreatic islet cells: an in vitro study clueing on islet graft immunoprotection in type 1 diabetes mellitus.
Basta G, Sarchielli P, Luca G, Racanicchi L, Nastruzzi C, Guido L, Mancuso F, Macchiarulo G, Calabrese G, Brunetti P, Calafiore R.
Alginate (AG)-based microcapsules may provide a selective permeable and biocompatible physical barrier to prevent islet graft (TX)-directed immune destruction. However, extent of the achieved immunoprotection will continue to be variable and unpredictable until the role of the individual mechanisms involved with TX-related inflammatory cell and immune reactivity are clarified. Macrophages (M) are believed to play a pivotal role in controlling the host/TX interaction and its consequences. We then have studied the effects of isolated rat M and their secretory products on allogeneic islets enveloped in variably sized and configured microcapsules, within in vitro mixed islet-M cocultures. In particular, we aimed to determine the sequence of immune or not immune specific cascade of early events that derive from such on interaction. One of the specific aims was to assess whether the membrane's physical intactness and conversely its even minimal rupture, along with the microcapsules' size (i.e., large vs. small) would significantly impact M reactivity and, thereby, the encapsulated islet viability and function. Special care was taken to evaluate extent of the elicited reactivity by meticulously monitoring cytokine, N2 derivative, and other proinflammatory protein curve profiles during the early M activation process. The study has preliminarily shown that, for equally formulated microcapsules, the capsular size and membrane's morphologic thoroughness are key to prevent M reactivity and possibly avoid the intracapsular islet cell damage. While elucidation of pathways involved with the encapsulated islet TX-directed host's responsiveness actually is in progress, it has clearly emerged that microcapsules should comply with well-defined physical properties and formulation specifications in order to obviate the primum movens of the inflammatory reaction process.

Scientific articles

8: Curr Drug Targets. 2004 Nov;5(8):735-44.
Peptide nucleic acids (PNA)-DNA chimeras targeting transcription factors as atool to modify gene expression.
Borgatti M, Finotti A, Romanelli A, Saviano M, Bianchi N, Lampronti I, Lambertini E, Penolazzi L, Nastruzzi C, Mischiati C, Piva R, Pedone C, Gambari R.
Peptide nucleic acids (PNAs)-DNA chimeras have been recently described as DNA mimics constituted of a part of PNA and of a part of DNA. We have demonstrated that double stranded molecules based on PNA-DNA chimeras bind to transcription factors in a sequence-dependent manner. Accordingly, these molecules can be used for transcription factor decoy (TFD) pharmacotherapy. Effects of double stranded PNA-DNA chimeras targeting NF-kappaB and Sp1 were determined on in vitro cultured human cells and were found to be comparable to those observed using double-stranded DNA decoys. The TFD molecules based on PNA-DNA chimeras can be further engineered by addition of short peptides facilitating cell penetration and nuclear localization. Therefore, these engineered molecules could be of great interest for in vivo experiments for non-viral gene therapy of a variety of diseases, including neoplastic and viral diseases, for which the TFD approach has been already demonstrated as a very useful strategy.

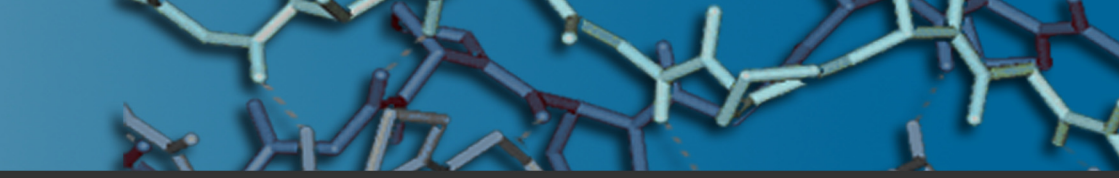
9: J Biomed Sci. 2004 Sep-Oct;11(5):697-704.
Complexation to cationic microspheres of double-stranded peptide nucleic acid-DNA chimeras exhibiting decoy activity.
Mischiati C, Sereni A, Finotti A, Breda L, Cortesi R, Nastruzzi C, Romanelli A, Saviano M, Bianchi N, Pedone C, Borgatti M, Gambari R.
The major aim of this paper was to determine whether cationic microspheres (CM), consisting of the permeable polymer Eudragit RS 100 plus the cationic surfactant dioctadecyl-dimethyl-ammonium bromide (DDAB(18)), could bind to double-stranded peptide nucleic acid PNA-DNA-PNA (PDP) chimeras exhibiting decoy activity against NF-kappaB transcription factors. Microspheres were produced by the 'solvent evaporation method' and centrifugation at 500, 1,000 and 3,000 rpm to obtain different-sized microparticles. Microsphere morphology, size and size distribution were determined by optical and electron microscopy observations. In order to determine their binding activity, double-stranded DNA-based and PDP-based decoy molecules were incubated with different amounts of microparticles in the presence of 100 ng of either (32)P-labeled DNA-DNA or DNA-PDP hybrid molecules or cold PDP-PDP hybrids. The complexes were analyzed by agarose gel electrophoresis. The resistance of (32)P-labeled DNA-DNA and DNA-PDP molecules in the presence of serum or cellular extracts was evaluated after binding to CM by gel electrophoresis analysis. DDAB(18) Eudragit RS 100 microspheres are able to bind to DNA-PDP and PDP-PDP hybrids, to deliver these molecules to target cells and to protect DNA-PDP molecules from enzymatic degradation in simulated biological fluids. In addition, when assayed in ex vivo conditions, DDAB(18) Eudragit RS 100 microspheres exhibited low toxicity. The results presented in this paper demonstrate that CM can be considered suitable formulations for pharmacogenomic therapy employing double-stranded PDP chimeras.



Scientific articles

10: Drug Deliv. 2004 Mar-Apr;11(2):83-8.
Liposomes containing distamycins: preparation, characterization and antiproliferative activity.
Cortesi R, Romagnoli R, Menegatti E, Esposito E, Cervellati F, Nastruzzi C.
This article describes the production and characterization of two liposome formulations containing antitumor drugs, namely distamycin A (Dist) and a new alkyl derivative of distamycin A (C16-Dist). Egg-PC/cholesterol liposomes (4:1 mol/mol) were prepared by reverse phase evaporation technique followed by extrusion through polycarbonate filters. The encapsulation efficiency was found to be almost complete for C16-Dist (99.8%), while native distamycin A showed a lower yield (19.0%). The in vitro antiproliferative activity of the distamycins-containing liposomes determined on human leukaemic K562 cells, was 11-fold and 8-fold higher for native and alkyl derivative distamycin A, respectively, compared with that of the corresponding free drugs. Liposomal formulations show an increase in the activity and specificity of distamycins in experimental antitumor therapy.

11: AAPS PharmSci. 2004 Jan 20;6(1):E2.
Formulations for natural and peptide nucleic acids based on cationic polymeric submicron particles.
Cortesi R, Mischiati C, Borgatti M, Breda L, Romanelli A, Saviano M, Pedone C, Gambari R, Nastruzzi C.
This article describes the production and characterization of cationic submicron particles constituted with Eudragit RS 100, plus different cationic surfactants, such as dioctadecyl-dimethyl-ammonium bromide (DDAB18) and diisobutylphenoxyethyl-dimethylbenzyl ammonium chloride (DEBDA), as a transport and delivery system for DNA/DNA and DNA/peptide nucleic acid (PNA) hybrids and PNA-DNA chimeras. Submicron particles could offer advantages over other delivery systems because they maintain unaltered physicochemical properties for long time periods, allowing long-term storage, and are suitable for industrial production. Submicron particles were characterized in terms of size, size distribution, morphology, and zeta potential. Moreover, the in vitro activity and ability of submicron particles to complex different types of nucleic acids were described. Finally, the ability of submicron particles to deliver functional genes to cells cultured in vitro was determined by a luciferase activity assay, demonstrating that submicron particles possess superior transfection efficiency with respect to commercially available, liposome-based transfection kits.



Scientific articles

12: Biomaterials. 2003 Aug;24(18):3101-14.
Multifunctional microcapsules for pancreatic islet cell entrapment: design, preparation and in vitro characterization.
Luca G, Basta G, Calafiore R, Rossi C, Giovagnoli S, Esposito E, Nastruzzi C.
Great advances in cell transplantation have been made, including the recent, remarkable success in pancreatic islet transplantation for the treatment of type 1 diabetes mellitus. Unfortunately, the transplanted cells are very susceptible to oxidative stress that cause severe damage to either allo- or xenogeneic islets upon graft in diabetic patients. Consequently, the transplanted islet functional life span is significantly shortened. The aim of this study was to examine the possible effects of antioxidants on in vitro cultured adult rat islets, and to evaluate the effects of a prolonged-release formulation, in form of cellulose acetate (CA) microspheres, on Vitamin D(3) activity. Isolated rat islets, both free and entrapped in microspheres were treated with Vitamin D(3). The effects of the vitamin were studied at 3, 6 and 9 days of in vitro cell culture. According to insulin secretory patterns, treatment with Vitamin D(3) of both free and CA entrapped microspheres, increased the insulin output as compared to untreated controls. Such positive effects were confirmed under islet static incubation with glucose at day 6. These results suggest that pancreatic islets can be advantageously treated with anti-oxidising vitamins before implantation, and speculatively, with the help of special delivery systems, throughout the islet cell life span, in the post-transplant time period.

13: Drug Deliv. 2003 Jul-Sep;10(3):139-49.
Aminated polysaccharide microspheres as DNA delivery systems.
Constantin M, Fundueanu G, Cortesi R, Esposito E, Nastruzzi C.
This article describes the production and characterization of cationic microparticles based on pullulan and starch for the delivery of nucleic acids. The microparticles were prepared by chemically cross-linkinking of a polymer solution dispersed in organic phase, followed by amination with N, N-diethyl-2-chloroethyl amine hydrochloride, or N-glycidyl-N,N-dimethyl-N methylammonium chloride. The association of desoxyribonucleotide (DNA) with positively charged microparticles was determined. The association capacity and the affinity of microspheres for DNA were investigated as a function of type of polysaccharide, content and basicity of the amino groups. It was found that the both types of carriers synthetized display a high affinity for defibrotide due to the high porosity of polysaccharide microspheres (PMs). The in vitro release kinetics from microspheres showed an initial fast release of DNA (30 min) followed by slower release rate over 14 days. DNA release was influenced by the ionic strength of the receiving fluid. In addition, DNA release was slightly more rapid from pullulan than from starch complexes. DNA stability studies were performed by agarose gel, indicating no degradation even after 14 days. All the produced cationic microspheres were able to quantitatively load DNA. The release of DNA from PMs was strongly affected by the ionic strength of the receiving fluid. Finally, agarose gel electrophoresis of DNA released from microspheres indicated that no DNA degradation occurs even after 14 days of release from PMs.

Scientific articles

14: J Cosmet Sci. 2003 May-Jun;54(3):239-50.
Diffusion of preservatives from topical dosage forms: a comparative study.
Esposito E, Bortolotti F, Nastruzzi C, Menegatti E, Cortesi R.

A study of the diffusion of parabens from topical formulations is presented here. In particular, four different topical formulations, namely, a water-in-oil emulsion, an oil-in-water emulsion, and two hydrophilic gels (Pemulen gel and Carbopol gel) were produced, containing a mixture of three common parabens, namely, methylparaben (MP), ethylparaben (EP), and propylparaben (PP). An analytical method based on liquid extraction, followed by reversed-phase HPLC for the quantitative determination of MP, EP, and PP, was developed. The method allowed good separation of paraben mixtures and high percentages of recovery (> than 97%). The diffusion kinetics of parabens from the produced formulations was determined by an in vitro system based on a Franz cell assembled with a synthetic membrane, followed by a reversed-phase HPLC analytical method. The comparative study demonstrated that, in the case of emulsions, diffusion coefficients are a function of the substituent of preservatives: the higher the solubility, the higher the diffusion of parabens. On the contrary, in the case of the hydrophilic gels, the higher the parabens solubility, the lower the diffusion coefficients. The method described here could represent a means of controlling the extent of diffusion of parabens from topical formulations in order to minimize percutaneous absorption and to control the availability of microbes.

Scientific articles

15: Oncol Res. 2003;13(5):279-87.
Resistance of decoy PNA-DNA chimeras to enzymatic degradation in cellular extracts and serum.
Borgatti M, Romanelli A, Saviano M, Pedone C, Lampronti I, Breda L, Nastruzzi C, Bianchi N, Mischiati C, Gambari R.

Double-stranded molecules based on peptide nucleic acids (PNAs)-DNA chimeras carrying binding sites for known transcription factors could be of great interest in decoy pharmacotherapy of neoplastic diseases. For instance, decoy molecules recognizing Sp1 and NF-kappaB transcription factors were found to inhibit tumor cell growth and invasion activity. In this respect, we have recently found that double-stranded PNA-DNA chimeras carrying NF-kappaB binding sites inhibit the binding of NF-kappaB p52 and p50 transcription factors to target DNA molecules. In this article we determined the resistance of double-stranded decoy molecules based on PNA-DNA chimeras to exonucleases (both 3'-->5' and 5'-->3' exonucleases), endonucleases, and 5'-phosphatases. In addition, we performed experiments aimed at determining the resistance of these molecules in cellular extracts and serum. Finally, we used liposomes as protective agents in experimental conditions in which the decoy molecules employed were found to be unstable (high concentrations of enzymes, cellular extracts, or serum). The results obtained demonstrated that decoy molecules based on PNA-DNA chimeras are more resistant than DNA-based decoys to exo- and endonucleases, serum, and cellular extracts. In addition, the resistance of DNA/PNA hybrids in the presence of high concentrations of serum and cellular extracts was increased after complexation to cationic liposomes, due to the fact that double-stranded PNA-DNA-PNA chimeras bind to these delivery systems. The results obtained in the present study support the proposal of molecules based on PNA-DNA chimeras for an efficient decoy treatment of tumor cells both in vitro and in vivo.

Scientific articles

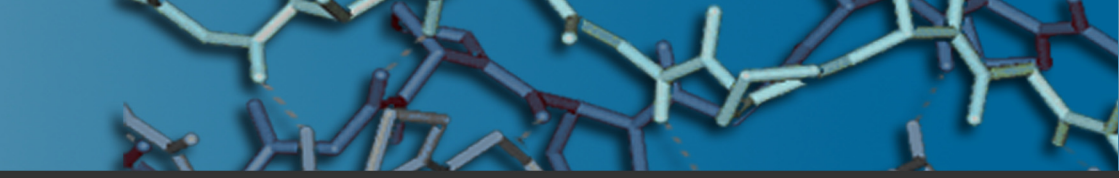
16: Int J Pharm. 2002 Aug 21;242(1-2):329-34.
Spray dried Eudragit microparticles as encapsulation devices for vitamin C.
Esposito E, Cervellati F, Menegatti E, Nastruzzi C, Cortesi R.
The aim of the present paper was to study production of methacrylate microparticles for the delivery (administration) of ascorbic acid via the oral route. Vitamin C is an important antioxidant that may be involved in the reduction of the risk of certain types of cancer, such as colorectal cancer. As polymers different acrylic compounds were considered, namely Eudragit RL, L and RS. Spray-drying was used as preparation method of vitamin C/Eudragit microspheres. Microspheres were first characterized by size and morphology by scanning electron microscopy, then in vitro release kinetics by mean of dialysis method were studied. Although the produced microparticles were unable to slow down the release of the drug with respect to the free form of ascorbic acid, these microspheres showed a good morphology and size distribution that permit to propose them as candidate for the delivery of vitamin C as associated therapy in the treatment of colorectal cancer by oral route.

17: Biochem Pharmacol. 2002 Aug 15;64(4):609-16.
Cationic liposomes as delivery systems for double-stranded PNA-DNA chimeras exhibiting decoy activity against NF-kappaB transcription factors.
Borgatti M, Breda L, Cortesi R, Nastruzzi C, Romanelli A, Saviano M, Bianchi N, Mischiati C, Pedone C, Gambari R.
Peptide nucleic acids (PNAs) have been recently proposed as useful molecules in pharmacogenetic therapy, especially due to the fact that they show a very high stability with respect to DNA and RNA. However, PNAs are not efficient decoy molecules, are characterized by negligible cell internalization and low solubility and are not suitable to be delivered by liposomes. With respect to the biological activity of PNA-based molecules, PDP deserve great consideration, due to the fact that they exhibit high levels of solubility, and are expected to be resistant to proteinases and exonucleases. In this manuscript we determined whether double-stranded molecules based on PNA-DNA chimeras containing NF-kappaB binding sites, exhibit decoy activity against NF-kappaB transcription factors. In addition, we determined whether they can be complexed by cationic liposomes. The results obtained demonstrated that hybrids based on PNA-DNA chimeras are powerful decoy molecules against NF-kappaB p52 transcription factor. In addition, we found that cationic liposomes can be proposed for in vitro delivery to target cells of these decoy molecules. The results presented in this paper are thus of practical importance, since the simplicity and the versatility of the cationic liposome technology have made cationic liposomes useful nonviral gene delivery systems for human gene therapy.

Scientific articles

18: Biomaterials. 2002 Jun;23(11):2283-94.
Production of lipospheres as carriers for bioactive compounds.
Cortesi R, Esposjto E, Luca G, Nastruzzi C.

Aim of the present paper was to investigate the influence of preparation parameters on the production of lipospheres (LS) for drug delivery. LS composed of triglycerides and monoglycerides were alternatively produced by melt dispersion technique, solvent evaporation or w/o/w double emulsion method. The influence of preparation parameters, such as (a) type and amount of lipids, (b) presence and concentration of surfactants, (c) stirring speed and (d) type of stirrer was studied. In the case of LS prepared by melt dispersion, the use of a lipid composition of cetyl alcohol/cholesterol (2:1, w/w), a 5% (w/w) gelatine solution (50 bloom grades) and 1000 rpm stirring speed resulted in the production of spherical particles, with high percentage of recovery (82%, w/w) a mean diameter of 80 microm and a narrow size distribution. In the case of LS prepared by solvent evaporation, the best results in terms of LS morphology, recovery and size distribution were obtained by the use of a lipid composition of tristearin/monostearate (66:34, w/w), a 1% (w/w) PVA solution, a 750 rpm stirring speed and a 55 mm three-blade turbine rotor. The solvent evaporation method resulted in the production of LS characterised by a smaller size (20 microm mean diameter) but poor mechanical properties with respect to particles with the same composition obtained by the melt dispersion technique (170 microm mean diameter). The use of a combination of lipids and a methacrylic polymer (Eudragit RS 100) overcame this problem, resulting in the production of spherical particles, with a narrower size distribution and good mechanical properties. Two lipophilic drugs, such as retinyl acetate and progesterone, and one hydrophilic drug, sodium cromoglycate (SCG), were encapsulated in LS as model compounds. Lypophilic drugs displayed satisfactory encapsulation efficiencies (over 70% w/w), while SCG was very scarcely encapsulated (about 2% w/w). To solve this drawback, the use of a w/o/w double emulsion strategy was proposed, enabling to increase the encapsulation of SCG up to 50% w/w. Finally, in vitro drug release studies were performed, showing that all drugs were released in a control manner. In particular. the retinyl acetate release efficacy within the first 8 h was 27% of the total amount of the drug, while in the same period, the amount of progesterone released was 63%. With regard to SCG containing LS, the release of the drug was largely influenced by the type of stabiliser of the primary emulsion, in any case the SCG release reached the 100% of the total amount of drug after 5 h from the beginning of the experiment.



Scientific articles

19: Ann N Y Acad Sci. 2001 Nov;944:160-79.
Pectin-based microspheres: a preformulatory study.
Esposito E, Cortesi R, Luca G, Nastruzzi C.
This paper reports on (a) the production of pectin microspheres and (b) the influence of different experimental parameters and ionic crosslinking on morphological and dimensional characteristics of pectin microspheres. Morphological and dimensional characteristics of pectin were analyzed as a function of the type of pectin, the dispersing phase, the stirring speed, and the emulsifying agent. Crosslinking by calcium chloride and the encapsulation of antibiotics (i.e., metronidazol and tetracycline) gave particles morphologically similar to empty particles but with slower swelling kinetic.

20: Drug Discov Today. 2001 Sep 1;6(17):893-904.
Delivery systems for DNA-binding drugs as gene expression modulators.
Cortesi R, Nastruzzi C.
Despite the large number of publications describing the synthesis and physicocharacterization of the binding between drugs and DNA, relatively few examine drug delivery systems (DDSs) for these molecules. The aim to find DDSs for DNA-binding drugs (DBDs) was prompted mainly to reduce the toxicity and/or enhance the tumor specificity of systemically administered drugs. With this in mind, we have reviewed the biological effects of some DBDs that are currently used as antitumor drugs and describe a brief selection of DDSs currently in clinical trials or on the market.

21: AAPS PharmSciTech. 2001 Aug 26;2(3):E15.
Improved function of rat islets upon co-microencapsulation with Sertoli's cells in alginate/poly-L ornithine.
Luca G, Calafiore R, Basta G, Ricci M, Calvitti M, Neri L, Nastruzzi C, Becchetti E, Capitani S, Brunetti P, Rossi C.
The purpose of this study was to assess whether Sertoli's cells would improve functional performance of homologous pancreatic islets within microcapsules. Purified rat Sertoli's cells were co-enveloped with islets in microcapsules that had been fabricated with alginic acid and poly-L-ornithine. Confocal laser microscopy was used to determine any mitogenic effects of Sertoli's cells on islets beta-cells. Insulin secretion from islets, with or without Sertoli's cells, was examined, and grafts of Sertoli's cells with islets in microcapsules into diabetic mice were carried out. Co-incubation of Sertoli's cells with islets resulted in a significant increase in the islet beta-cell mitotic rate, which was coupled with significantly higher insulin release under glucose stimulation, as compared to controls. Grafts of co-microencapsulated Sertoli's cells with islets resulted in prolongation of the achieved normoglycemia in the animals receiving Sertoli's cells with islets as compared to controls that received islets only. Sertoli's cells do promote mitogenic activities upon in vitro co-incubation with islets, whose in vitro functional and in vivo post-transplant consequences were evident. Sertoli's cells could, therefore, be co-microencapsulated with islets for transplantation in diabetic recipients.

Scientific articles

22: Int J Pharm. 2001 May 7;218(1-2):13-25.
Acrylic microspheres for oral controlled release of the biguanide buformin.
Fundueanu G, Mocanu G, Constantin M, Carpov A, Bulacovschi V, Esposito E, Nastruzzi C.
Spherical microparticles based on methacrylic acid-methyl methacrylate copolymer have been developed. The method chosen for the preparation of such microparticles was suspension radical copolymerization of acrylic comonomers in the presence of the ethyleneglycol dimethacrylate as crosslinking agent. The microparticles obtained were characterised by inverse size exclusion chromatography, scanning electron microscopy, swelling degree and exchange capacity. The porous volume of the microspheres ranged from 0.086 ml/g for the microparticles produced by a methacrylic acid/methyl methacrylate ratio of 1/3 and a 10% degree of crosslinking, to 8.57 ml/g for the microparticles produced by a methacrylic acid/methyl methacrylate ratio of 3/1 and 2% degree of crosslinking (in 0.1 N NaCl in phosphate buffer pH 7.4). Also the pore diameter of the swollen microparticles ranged from a few to 120 A. Buformin tosylate - a classical hypoglycaemic drug - was included in the polymer network of the microparticles during the polymerization process. Due to the water solubility of the drug and its low solubility in the organic phase, the entrapment yield did not exceed 15%. However the amount of encapsulated drug as well as the drug released from the microparticles, was dependent on the methacrylic acid/methyl methacrylate ratio, the degree of crosslinking and solvent/comonomers ratio.

23: J Steroid Biochem Mol Biol. 2000 Dec 15;75(2-3):121-8.
Modulation of estrogen receptor gene transcription in breast cancer cells by liposome delivered decoy molecules.
Piva R, del Senno L, Lambertini E, Penolazzi L, Nastruzzi C.
It is well known that breast carcinomas without estrogen receptor (ER) have a poor prognosis and do not respond to antiestrogenic therapy. In analyzing the question of the lack of ER gene expression, we have considered the possibility to modify the ER gene expression by transfecting ER-negative breast cancer cells with a polymerase chain reaction product mimicking a putative negative regulatory region (--3258/--3157) inside the P3 ER gene promoter. Here we have demonstrated the efficacy of the selected sequence used as a decoy molecule in restoring the ER gene transcription. When this DNA was complexed and delivered by cationic liposomes (PC:DOTAP) a significant increase in the decoy effect was obtained. Breast cancer cells receiving the combination treatment responded substantially better to reactivation of quiescent ER gene than cells that had received DNA with calcium phosphate. This information may be useful for a series of in vitro transfections and also for in vivo application of the decoy strategy that is a potential therapeutic tool to control disease-related genes such as ER gene in breast cancer.

Scientific articles

24: Diabetes Nutr Metab. 2000 Dec;13(6):301-7.
Effects of anti-oxidizing vitamins on in vitro cultured porcine neonatal pancreatic islet cells.
Luca G, Nastruzzi C, Basta G, Brozzetti A, Saturni A, Mughetti D, Ricci M, Rossi C, Brunetti P, Calafiore R.
Oxidative stress may cause severe cellular damage to both allo- and xeno-transplanted islets, additional to islet graft directed immunity, in diabetic patients. We thus aimed to examine the effects of antioxidants on in vitro culture-maintained, neonatal porcine cell clusters (NPCCs). NPCCs were treated with antioxidants (vitamins D3 and E) by a certain time of their maturation and differentiation process. Insulin recovery showed that both vitamins D3 and E, unlike untreated controls, resulted in preservation of the islet function for significantly long periods of time. Such effects were also confirmed during NPCCs in vitro static incubation with high glucose. Furthermore, morphologic examination of NPCCs demonstrated that at 16 days of cell culture beta-cell clusters were significantly larger and more intact when exposed to the vitamins as compared to controls. According to these preliminary results, because the employed vitamins, known to retain anti-oxidizing properties, seemed to clearly improve NPCCs morphology and function, they may represent a potentially useful tool for islet culture maintenance in the pre-transplant time period.

Scientific articles

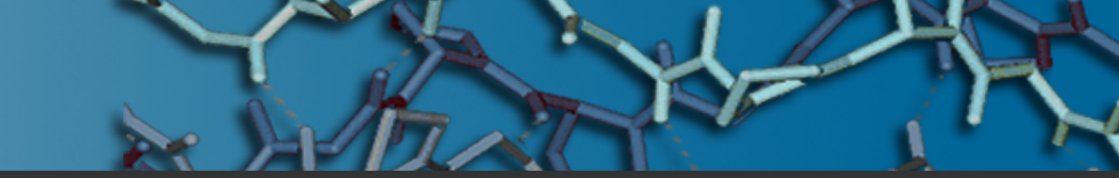
25: J Biomed Mater Res. 2000 Oct;52(1):40-52.
Rheologic and NMR characterization of monoglyceride-based formulations.
D'Antona P, Parker WO, Zanirato MC, Esposito E, Nastruzzi C.

This paper describes the production and characterization of semi-solid formulations based on monoglycerides from canola oil and water as drug-delivery systems. In order to obtain new formulations with different characteristics in terms of viscosity, bioadhesiveness, and solubilization capacity, a third component was added to the monoglyceride-water system. Nine excipients were tested, namely soy oil, isopropylmyristate, isopropylpalmitate, tripalmitin, tristearin, glyceryl monostearate, glycerol, propylene glycol, and ethanol. In particular, the effect of each excipient on the viscosity and stability of the formulation was investigated. It was found that ethanol dramatically influenced the viscosity of the monoglyceride-water system, resulting in the formation of stable forms. In addition, ethanol suitably could be used for the solubilization of water-insoluble lipophilic drugs. This promising ternary system was characterized by microscopic, NMR spectroscopic, and rheologic techniques. (1)H and (13)C NMR studies were made of Myverol to verify the molecular structure and isomer distributions of this commercial monoacylglycerol mixture. The microstructure of an isotropic solution consisting of Myverol [1.8% (w/w)], ethanol (42.9%), and water (55.3%) was studied by the multi-component self-diffusion NMR method. From the self-diffusion coefficient (D) of the monoglycerides (8.8 x 10-11 m(2)/s), an "apparent spherical hydrodynamic radius" of ca. 2.48 nm was calculated for the micellar aggregate. A nearly spherical shape is consistent with these values since the extended hydrocarbon chain of the longest monoglyceride (17 carbons) is ca. 2.2 nm long. The D's of water and ethanol reveal they do not associate (no attractive nonbonding interactions) appreciably with the fatty acid micelles.

Scientific articles

26: J Control Release. 2000 Aug 10;68(2):237-49.
Liposomes as carriers for DNA-PNA hybrids.
Nastruzzi C, Cortesi R, Esposito E, Gambari R, Borgatti M, Bianchi N, Feriotto G, Mischiati C.
Peptide nucleic acids (PNAs) are DNA mimics composed of N-(2-aminoethyl)glycine units. This structure gives to PNAs (a) resistance to DNases and proteinases, (b) capacity to hybridize with high affinity to complementary sequences of single-stranded RNA and DNA, and (c) capacity to form highly stable (PNA)(2)-RNA triplexes with RNA targets. Furthermore, DNA-PNA hybrid molecules are capable to reversibly interact with DNA-binding proteins, being therefore of interest for studies on regulation of gene expression by the decoy approach. The major conclusion of this paper is that cationic liposomes are able to efficiently complexate DNA-PNA hybrid molecules and mediate their binding to target cells. Our results are of some interest, since, unlike commonly used nucleic acids analogs, PNA oligomers are not taken up spontaneously into the cells. In addition, they are not suitable for an efficient delivery with commonly used liposomal formulations. Transfection of PNA-DNA hybrid molecules to in vitro cultured cells could be of great interest to determine the applications of these new reagents to experimental alteration of gene expression.

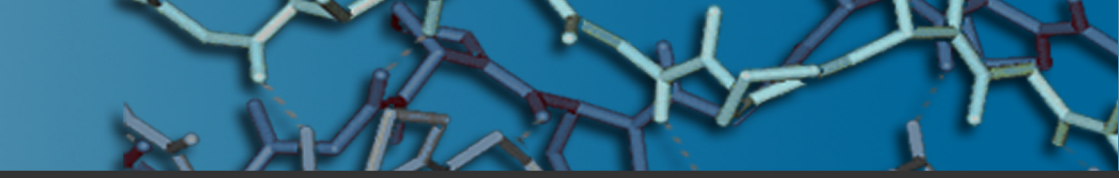
27: J Agric Food Chem. 2000 Aug;48(8):3572-5.
In vitro antiproliferative activity of isothiocyanates and nitriles generated by myrosinase-mediated hydrolysis of glucosinolates from seeds of cruciferous vegetables.
Nastruzzi C, Cortesi R, Esposito E, Menegatti E, Leoni O, Iori R, Palmieri S.
A comparison of the effect of isothiocyanates and nitriles derived from some glucosinolates, namely, epi-progoitrin, sinalbin, glucotropaeolin, glucocheirolin, and glucoraphenin, on human erythroleukemic in vitro cultured cells was studied. Many studies have in fact evidenced that a consumption of vegetable containing glucosinolates could reduce the development of colorectal cancer. In the experimental conditions used, the production of isothiocyanates and nitriles from glucosinolates is almost quantitative as confirmed by HPLC or GC-MS analysis. The obtained results demonstrated that in general nitriles are considerably less potent than the corresponding isothiocyanates in inhibiting cancer cell growth. Particularly, the isothiocyanates inhibitory activity on K562 cells growth is higher in the case of products derived from epi-progoitrin, glucotropaeolin, glucoraphenin, and glucocheirolin; while for nitriles the higher activity in inhibiting K562 cells growth is showed by sinalbin-derived product. Considering the antiproliferative activity found for isothiocyanates and nitriles, further studies will be aimed to the possible application of glucosinolate-derived products as chemopreventive cancer agents for the reduction of colorectal cancer.



Scientific articles

28: Antisense Nucleic Acid Drug Dev. 2000 Jun;10(3):205-15.
Effect of DNA complexation and freeze-drying on the physicochemical characteristics of cationic liposomes.
Cortesi R, Esposito E, Nastruzzi C.
We describe the use of saccharides, such as sorbitol, mannitol, sucrose, maltodextrin, and dextran, as cyoprotectants for freeze-drying cationic liposomes. Saccharides can protect liposomes either by interacting with phospholipid headgroups or by forming an amorphous glass surrounding the vesicles, thus preventing aggregation, mechanical rupture of membrane, fusion of liposomes, and drug leakage. We have particularly considered liposome characteristics, such as size, zeta potential, and ability in complexing DNA, before and after freeze-drying. Our study indicates that cationic liposomes are able to maintain liposome characteristics after lyophilization and rehydration and maintain the ability to complex DNA even if the strength of the interaction forces was of lower intensity with respect to liposomes before lyophilization.

29: AAPS PharmSciTech. 2000 May 6;1(2):E9.
Influence of formulation and process parameters on pellet production by powder layering technique.
Nastruzzi C, Cortesi R, Esposito E, Genovesi A, Spadoni A, Vecchio C, Menegatti E.
The goal of the present study was to evaluate the influence of the formulation and operating conditions on pellet preparation by pan technique. To this end, a new pelletization process, typified by the application of powdered drug on sugar-based cores using the GS coating system was studied. Inert cores were intermittently treated with micronized drug powder and adhesive solution. This treatment led to the formation of multiple layers of drug particles around an inert core resulting in the production of pellets that can further be coated by different polymers to obtain modified release formulations. Different procedures have been used to evaluate a series of important parameters such as initial core weight; speed of powder application; speed, type, and position of the atomizers; atomization degree; temperature; and air cap. Good yield of drug layering was obtained by adjusting the quantity of both the drug powder to apply and the binder solution. Pellets obtained following the optimal operating conditions (defined in a pre-formulation study) were film coated with the acrylic polymer Eudragit L30D in order to produce a model formulation consisting of enteric polymer-coated pellets containing ibuprofen. During its preparation, the formulation showed no degradation of the drug; moreover, a low percentage of residual humidity was obtained, indicating that this system is very efficient for the production of highly stable formulations. This study showed the good performance of the GS automated pan-coating system in obtaining enteric coated pellets prepared by powder layering technique using aqueous solutions.

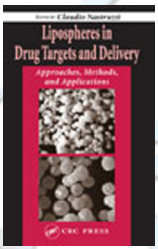


Scientific articles

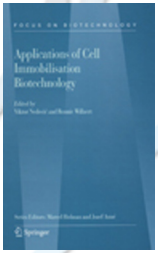
30: AAPS PharmSciTech. 2000 Mar 3;1(1):E2.
Liposome-based formulations for the antibiotic nonapeptide Leucinostatin A: Fourier transform infrared spectroscopy characterization and in vivo toxicologic study.
Ricci M, Sassi P, Nastruzzi C, Rossi C.
Leucinostatin-A is a nonapeptide isolated from Paecilomyces marquandii, Paecilomyces lilacinus A257, and Acremonium sp., exerting remarkable phytotoxic, antibacterial (especially against Gram-positive) and antimycotic activities. With the aim to find alternative formulation for in vivo administration, a number of Leucinostatin-A-loaded liposomal formulations have been prepared and characterized. Both large unilamellar vesicles and multilamellar vesicles consisting of synthetic and natural lipids were evaluated. In addition, to determine the nature of peptide-membrane interactions and the stability of liposomes loaded with Leucinostatin-A, a Fourier Transform Infrared Spectroscopy study was performed. The results suggest that the mode of interaction of the peptide is dependent on its concentration, on bilayer fluidity, and on liposome type. Finally, the LD50 of both free and liposome-delivered Leucinostatin-A was determined in mice. These results suggest that the incorporation of Leucinostatin-A into liposomes may result in decreased Leucinostatin-A toxicity, as the intraperitoneal administration of Leucinostatin-A-loaded liposomes reduced the LD50 of Leucinostatin-A 15-fold.

31: Pharm Dev Technol. 2000;5(2):267-78.
Production of Eudragit microparticles by spray-drying technique: influence of experimental parameters on morphological and dimensional characteristics.
Esposito E, Roncarati R, Cortesi R, Cervellati F, Nastruzzi C.
The aim of this study was to evaluate the influence of operating parameters on the characteristics of methacrylate microparticles prepared by spray-drying technique. Eudragit microparticles were prepared by a spray-drying method. The influence of different experimental parameters (i.e., solvent, feed rate, air flow rate, air-drying temperature, and aspiration flow rate) on microparticle morphology, size distribution, and recovery was studied. In addition, different Eudragit types and Eudragit RS concentrations were employed. Optical and electron microscopy were employed to analyze microparticle morphology and dimensional distribution. Finally, prednisolone as model drug was encapsulated in Eudragit RS microparticles. Low feed rate values yielded the best results in terms of microparticle morphology. Changes in nebulizing air flow did not result in a corresponding effect on microparticle characteristics. An increase of air-drying temperature resulted in a reduction of microparticle dimension and recovery. A low flow of drying air was preferable because this resulted in microparticles with an optimal morphology. The polymer concentration affected both morphology and dimensions of microparticles. The encapsulation of prednisolone led to good incorporation efficiencies without altering percentage of recovery, morphology, and mean dimension of the microparticles. The selection of appropriate parameters yielded spray-dried Eudragit RS microparticles characterized by good morphology and narrow dimensional distribution

Books, book chapters & special issue



Liposphere in Drug Targets and Delivery: Approaches, Methods, and Applications
Claudio Nastruzzi, University of Perugia, Italy
ISBN: 0849316928
Publication Date: 11/29/2004
Discusses innovative methods of delivering bioactive substances to different systems.
Presents lipospheres as a technical solution to problems associated with controlled release of drugs.
Covers lipospheres as carriers for vaccines
Provides procedures for specific applications and biological systems.



BIO-ARTIFICIAL ORGANS, the example of artificial pancreas
C. Nastruzzi, G. Luca, G. Basta, R. Calafiore.
Part 1, Tissue engineering, pages 17-35

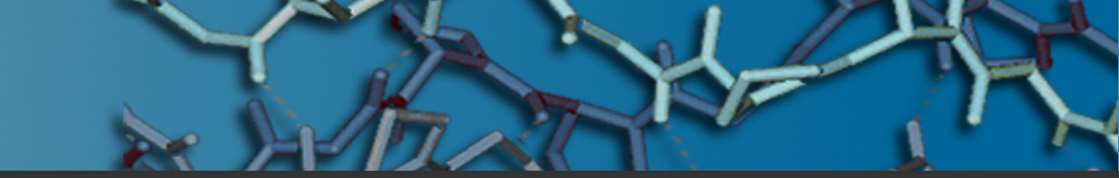
Applications of cell immobilisation biotechnology
edited by: V. Nedivic and R.
Springer Ed.

New trends in bioencapsulation 2
edited by: Claudio Nastruzzi

MINERVA BIOTECNOLOGICA
Volume 17- No. 4 , december 2005
pages 259-269

New trends in bioencapsulation 2
edited by: Claudio Nastruzzi.

MINERVA BIOTECNOLOGICA
Volume 18- No. 1 , march 2006
pages 57-63



Partecipazioni a congressi

Production and characterization of lipidic microspheres for biopharmaceutical application on a 'Lab-on-a chip' .
A.Tosi, L. Bilancetti, S. Di Croce, G. Luca, C. Nastruzzi.
XIth International Workshop on Bioencapsulation.
Vitoria-Gasteiz, Spain - September 24-26, 2004.

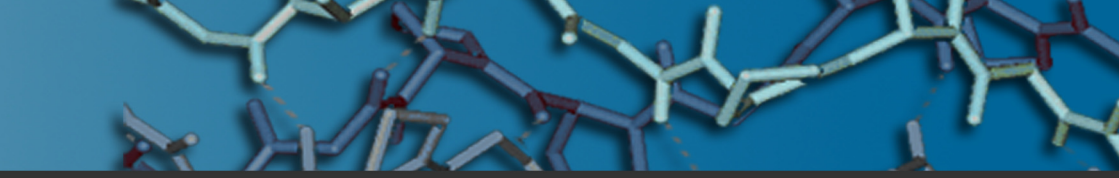
Multicompartimental microdevices for islet transplantation.
G. Luca, A. Tosi, L. Bilancetti, S. Di Croce, R. Calafiore, C. Nastruzzi.
7th International Congress of the Cell Transplant Society.
Boston,USA-November 17-20,2004.

Cellulose acetate microparticles for lab-on-a-chip applications.
S. Di Croce, A. Tosi, L. Bilancetti, S. Mazzitelli, N. Bozzuto, C. Nastruzzi.
XIIIth International Workshop on Bioencapsulation.
Kingston, Canada-June 24-26, 2005.

Design and production of alginate-based microdevices for Sertoli's cells encapsulation.
L. Bilancetti, A. Tosi, S. Di Croce, S. Mazzitelli, N. Bozzuto, G. Luca, R. Calafiore, C. Nastruzzi.
XIIIth International Workshop on Bioencapsulation.
Kingston, Canada-June 24-26, 2005.

Tripalmitin-based cationic lipospheres: preparation, characterization and in Lab-on-a-chip applications.
A. Tosi, S. Mazzitelli, N. Bozzuto, B. Bertini, G. Luca, C. Nastruzzi
9th European Symposium on Controlled Drug Delivery.
Noordwijk aan Zee, The Netherlands -April 5-7, 2006.

Optimization of a solvent evaporation method for preparation of cellulose acetate microparticles for Lab-on-a-chip applications and characterization thereof.
A. Tosi, S. Mazzitelli, N. Bozzuto, B. Bertini, C. Nastruzzi
9th National Congress on Molecular Biotechnology.
Torino, Italy 7-9 September 2006.



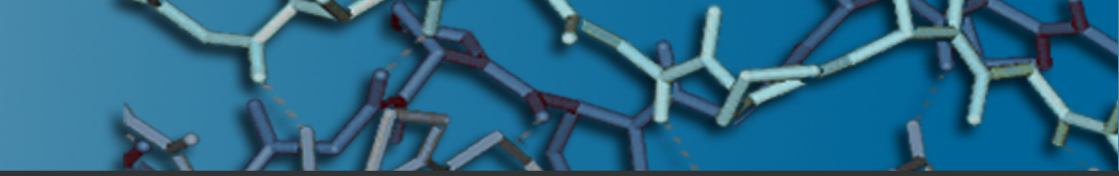
Partecipazioni a congressi

Agarose microparticles: influence of manufacturing parameters on the size characteristics.
B. Bertini, A. Tosi, S. Mazzitelli, N. Bozzuto,C. Balestra, G. Luca, C. Nastruzzi.
20th European Conference on Biomaterials.
Cité des Congrès, Nantes, (France), 27 September - 1 October 2006.

Preparation of agarose based microcapsules by emulsion and microdroplet generator techniques.
A. Tosi, S. Mazzitelli, N. Bozzuto, C. Balestra, B. Bertini, G. Luca, C. Nastruzzi
XIVth international workshop on Bioencapsulation.
Lausanne Switzerland 5-7 October 2006.

Functional properties and in vivo biocompatibility of microencapsulated Sertoli's cells.
G. Luca, M. Calvitti, R. Calafiore, A. Tosi, S., C. Nastruzzi
XIVth international workshop on Bioencapsulation.
Lausanne Switzerland 5-7 October 2006

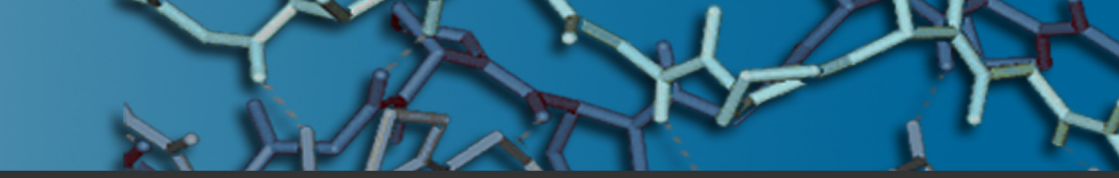
Encapsulation of Sertoli cells in agarose-based microdevices for nerve repair: preparation, characterization and in vitro biocompatibility.
S. Mazzitelli, C. Balestra, A. Tosi , N. Bozzuto, G. Luca, R. Calafiore and C. Nastruzzi
BioMed 2007 - The Fifth IASTED International Conference on Biomedical Engineering.
Innsbruck, Austria - February 14-16, 2007



Refeering

Il prof. Nastruzzi ha svolto e sta svolgendo attività di “refeering” per alcune delle più prestigiose riviste scientifiche nel campo della tecnologia farmaceutica e dei biomateriali.

-Journal of American Chemical Society
-Journal of Controlled Release
- European Journal of Pharmaceutics and Biopharmaceutics
-Journal of Microencapsulation
-AAPS PharmSciTech
-Journal Pharmaceutical Sciences
-Journal of Biomadical Materials Research
-Biomaterials
-Drug Development Industrial Pharmacy



Finanziamenti

Il Laboratorio di Biomateriali & Bioincapsulazione ha ricevuto finanziamenti da istituzioni pubbliche e private.

-Ministero dell'Università e della Ricerca Scientifica.
Progetti FIRB
Progetti COFIN

-Fondazione Cassa di Risparmio di Perugia.

-Regione Umbria.
Progetto Ponte sul Distretto

-Università degli Studi di Perugia.
Assegni di ricerca

-Progetti di collaborazione con aziende farmaceutiche nazionali ed internazionali.

-Progetti di collaborazione con aziende biotecnologiche internazionali.

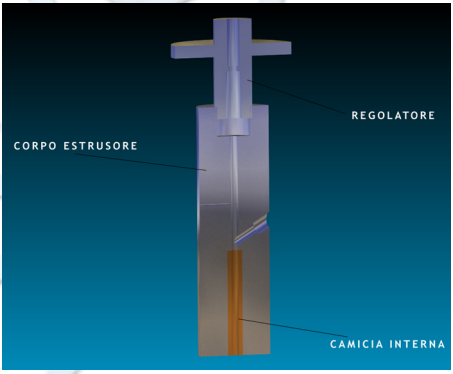
-Consorzio Interuniversitario Biotecnologie (CIB).
Finanziamento mobilità giovani ricercatori

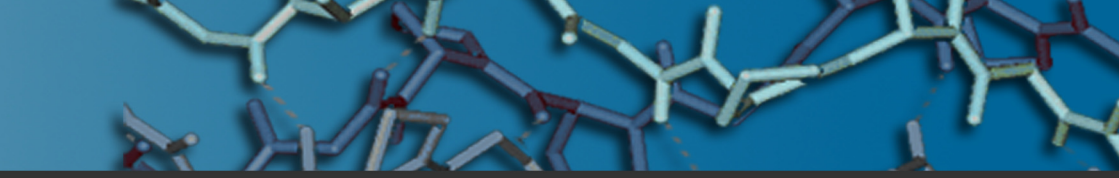
-Progetto comunità europea COST.
progetto COST 840: Bioencapsulation innovations and technologies
progetto COST 865: Bioencapsulation multiscale interaction analysis

-Short term Scientific Mission (STSM, COST 840 & 865).

Attività conto terzi

Il Laboratorio di Biomateriali e Bioincapsulazione svolge attività di consulenza per aziende del settore farmaceutico, dei biomateriali, cosmetico e alimentare. Sono stati sviluppati progetti a contratto per la preparazione di batch di prodotti fino all'impianto pilota.
Sono state inoltre sviluppate diverse strategie di incapsulazione che hanno permesso di ottenere prodotti microincapsulati con varie specifiche industriali.
Più recentemente é stato avviato un servizio di progettazione e disegno meccanico per la realizzazione di strumentazione da laboratorio per processi di bioincapsulazione. Siamo in grado di produrre modelli di oggetti tridimensionali molto rapidamente attraverso l'utilizzo di opportuni software CAD/CAM/CAE.





Contatti

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