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Surface Functionalization of Single Wall Short Carbon Nanotubes by PEG-PE Lipid-Core Polymeric Micelle for Nucleolin Aptamer Targted Tumor Delivery of Chemotherapeutic Agents

The effectiveness of a cancer therapeutic device is measured by its ability to reduce and eliminate tumors without damaging healthy tissues. It is possible by functionalizing the surface of nanocarriers with ligands such as antibodies or aptamers.

Aptamers are nucleic acid based ligands that fold into unique three-dimensional conformations, capable of binding to target antigens with high affinity and specificity and have many attributes that make them superior to antibodies for the molecular recognition. In the cancer arena, one of the most advanced aptamer is GRO that binds to the external domain of the membrane protein nucleolin, generally associated with poor clinical prognosis for some types of cancers.

Among the numerous delivery systems currently under investigations, carbon nanotube (CNT)-based drug delivery especially short single wall nanotubes (SWNT) has shown promise in various in-vitro and in-vivo experiments. CNTs have ultrahigh surface area ready for hydrophobic interactions that permits efficient loading of multiple molecules such as chemotherapeutic agents along the length of the nanotube sidewall. This could overcome the probelms of developing multidrug resistance and general toxicities.

In present study, we aimed to design, prepare and assess the properties of CNTs functionalized with GRO aptamer for targeted drug delivery to cancer cells.

GRO aptamer was formed and structured in KCl supplemented HEPES buffer (pH=7.4) and upon heat-denaturation and renaturation in 60C. The formation of unique homogeneous aptemer structure was determined by native PAGE in different thermal and buffer conditions. Thiolated GRO aptamers were reduced using DTT, purified by multiple stage ethyl acetate - buffer extraction and quantified using Ellman's reagent.

PEG-DSPE micelles (one fifth maleimide-functionalized) were prepared by reconstitution and sonication in HEPES buffer (pH=7.4) of lipid film formed following chloroform removal by purging N2 stream.

Thiolated aptamers were conjugated to PEG-DSPE micelles at 5:1 mole ratio through maleimide-thiol reaction (Ap-PEG-DSPE). Lipid core polymeric micelles composed of PEG-DSPE and Ap-PEG-DSPE was mixed with SWNT and sonicated intermittently in cool condition to disrupt SWNT bundles and coat them through surface adsorption. Alternatively, sodium deoxycholate covering SWNT was replaced by Ap-PEG-PE. Surface functionalized SWNTs were purified by ultrafiltration 30KDa at 12000 rpm. Doxorubicin is going to be loaded onto Apt-PEG-CNTs by simply mixing at various pH values overnight. Effect of pH on loading efficiency and release behavior will be measured by fluorimetry.

Formation of unique aptamer structure and thereupon its effectiveness was cation type dependent, but rapid and independent of renaturation thermal treatments as confirmed by native PAGE. Thiolated aptamers required reduction of disulfide bond by DTT and further purification as determined by Ellman's reagent. This resulted in about 90% reduced aptamer recovery. PEG-DSPE micelles formed at concentrations higher than 10µM with sizes in range of 40-60 nm. GRO thiolated aptamers were reacted at high yield with PEG-DSPE micelles at 1:10 ratio.

It is under investigation if protonation of doxorubicin at acidic pH could influence drug loading and release profile. This pH-dependency may favor maintaining doxorubicin loading on SWNT at physiologic pH and delivery to acidic environment of tumors. Besides, nanotechnology based targeted therapy with the internalizing receptor, nucleolin, could promote doxorubicin efficiency and reduce unspecific effects of not only doxorubicin, but also the cytotoxicity associated with SWNT solely or induced hyperthermia upon NIR radiation.

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Use of MRI and micro CT to monitor Rheumatoid Arthritis disease progression and regression, following treatment with nanocarrier encapsulated drug

Rheumatoid arthritis (RA) is a chronic, systemic, and progressive inflammatory disorder of the synovium, which is characterized by destruction of bone and cartilage. Outcome of RA is known to benefit greatly from early diagnosis, and timely, intensive therapy. The central aims of this study are to develop noninvasive imaging techniques for monitoring the disease progress and the effects of treatment, with drug loaded nanoparticles.

10 male DBA1 mice (10 weeks of age) were used for the Collagen Induced Arthritis model. Disease severity was clinically scored by joint examination on a scale 0 - 2. Animals were scanned at different time points of the disease progress, and were treated once with 10 mg/kg of saline as control, Dexamethasone as free drug and Dexamethasone-PEG-liposomes as nanocarrier drug. Animals were sacrificed in the end for histology. MRI examination was performed on a 6.3 T scanner (Bruker Biospin). We developed a novel radio-frequency coil set-up for in vivo MRI studies on RA. T1-weighted images (TR/TE=1000/10.2 ms) were recorded for different cross sections.

The area of the sagittal MRI images and micro CT of the mouse paws, acquired at different stages of the disease progress, showed very good correlation with the disease progress score. These encouraging preclinical results for monitoring the progress of the disease were used to evaluate anti-inflammatory therapy with MRI. A single dose of Dexamethasone did not result in large decrease of paw inflammation scores. However, the same single dose encapsulated in PEG-liposomes resulted in a complete disappearance of paw inflammation at day 5 post-treatment, whereafter inflammation increased again. The data in this study demonstrate the profound anti-inflammatory activity of Dexamethasone-PEG-Liposomes, where the reduction of paw inflammation was rapid and the therapeutic effect lasted more than a week. This indicates that the encapsulation of the drug in the nanocarrier system can strongly enhance its beneficial effect in RA.

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Functional motor recovery from brain ischemic insult by carbon nanotube-mediated siRNA silencing

Ischemic stroke accounts for 80% of all stroke insults and often leads to chronic functional limitations that adversely and long-lastly affect the ability of patients to movement. Physical and pharmacological rehabilitation strategies to reduce chronic impairment are often not sufficient to return to pre-lesion levels of performance of sensory and motor functions. In the past, gene therapy has been suggested to limit stroke derived anatomical and functional damage [1] and viral vector administration of genes into the affected area of the brain has been exploited. Carbon nanotubes have been recently proposed as novel nanomaterials that can offer significant advantages for the intracellular delivery of nucleic acids, such as siRNA. We have recently demonstrated in a proof-of-principle study [2] that amino-functionalized multi-walled carbon nanotubes (f-MWNT) can effectively deliver in vivo an siRNA sequence triggering cell apoptosis that results in human lung xenograft eradication and prolonged survival. Herein, we have investigated the use of the same functionalized carbon nanotubes material, to design a vector for non-viral delivery of small interference RNA (siRNA) directly into the brain (intracranial administration). This was based on the hypothesis that siRNA against neuronal Caspase-3 could rescue cells from stroke-induced apoptosis after injection of 60 picomoles of endothelin-1 (ET-1). ET-1 is a potent vasoconstrictor that when injected in the motor cortex (M1) induces stroke-like events that cause specific impairments of mobility performance. Using the skilled reaching test in rats, we demonstrate that gene silencing using CNT:siRNA vectors 24hr pre-endothelin administration reduces apoptosis in M1 and enhances motor skill recovery in rats after ischemic stroke.

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Magnetic properties and MRI contrast efficiency of novel ferrofluid-based nanoparticles

We have investigated novel ferrofluids based on superparamagnetic (SP) nanoparticles, having core and hydrodynamic diameters ranging from 3.8 to 15 nm and from 53 to 106 nm, respectively. Our samples which are made of biocompatible components, have shown low cellular toxicity1 and have the possibility of being functionalized for biomedical applications in the fields of diagnostics and therapy. We have here studied their efficiency as contrast agents in Magnetic Resonance Imaging (MRI). To this aim, the efficiency of the samples as a function of the magnetic core size has been investigated by performing 1H NMR relaxometry experiments. To cover the operating frequencies of most clinical Imagers, the proton spin-lattice relaxation time T1 and the spin-spin relaxation time T2 have been measured in the frequency range 10KHz-200MHz, and 4MHz-200MHz, respectively. The NMR relaxometry profiles show that the transverse relaxivity, the parameter which represents the contrast efficiency in the MRI, for some of our samples is comparable with or better than that of commercial SP compounds. The best efficiencies have been obtained in ferrofluids with larger magnetic core sizes. In vitro MRI experiments performed at 8.5 MHz have confirmed the relaxometric results, thus allowing us to propose our samples as novel negative MRI contrast agents.

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NEW VEHICLES FOR DRUG DELIVERY: MACROPOROUS SOLID FOAMS PREPARED FROM HIGHLY CONCENTRATED EMULSIONS

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The polymerization in the continuous phase of highly concentrated emulsions allows the preparation of solid foams with very high pore volume that can be used as drug delivery systems. The objective of this research has been the incorporation of model lipophilic and hydrophilic drugs (ketoprofen and clindamycin hydrochloride respectively) to polystyrene macroporous hydrophobic solid foams, previously prepared by a method based on the PIT emulsification method. Drug release to a receptor solution was studied by a molecular dialysis method.

To ensure homogeneous impregnation and drug delivery from the macroporous materials, the monoliths obtained were immersed in water/alcohol solutions (1:1 w/w) containing 2 %, 5% or 10 % of ketoprofen or clindamycin hydrochloride, respectively. The solid foams were then dried at 50ºC for 24 h after the incorporation of the drug, which was quantified by the weight increase of the materials.

Diffusion experiments were carried out in dialysis bags containing the macroporous materials with the drug and filled with PBS (pH=7.4), which was also used as a receptor media, or in a dissolution test equipment. Both, dialysis bags and membranes for dissolution test, were porous cellulose membranes with a molecular weight much bigger than the molecular weight of ketoprofen and clindamycin. The diffusion cell consisted on a cylindrical thermojacketed glass vessel, thermostated at 37ºC. The diffusion rate of the drug dialysis bags into the receptor was determined by measuring the amount of drug diffused as a function of time as determined by UV espectrofotometry (233 nm for ketoprofen and 210 nm for clindamycin hydrochloride).

The polystyrene materials used in this work as potential drug delivery systems contain macropores with a certain polydispersity, preserving the structure of the continuous phase of the emulsion which served as template for polymerisation.

The impregnation is the critical point of the process, since it depends on the size and shape of the monolith as well as on its weight. It has been observed that the most homogeneous impregnation results are obtained by the immersion method of the solid foams in a solution with the drug and the latter drying.

Weight uptake by the monolith disks increases linearly with the concentration of drug in the aqueous solution from 23% to 67% and weight uptake in solutions containing 2% to 10% of drugs, respectively. However, the amount of active principle in the monolith does not always seem to be related to the drug release, and varies from 80,8 % to 93,4 % at 24 h.

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sPLA2-SUSCEPTIBLE ANTICANCER DOUBLE LIPID PRODRUGS
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The use of liposomal drug delivery systems for cancer treatment attracted a lot of interests after the description of the enhanced permeability and retention phenomenon (EPR). However, only a few products have made it to the Clinique due to various complications. On the one side, the biophysical chemistry of lipid bilayers is complex. On the other side the dilemma between making stable liposomal encapsulations outside the target area and providing for drug release at the target has been difficult to resolve.
The escape of encapsulated drugs from the liposomes by passive diffusion often leads to suboptimal drug concentrations in the cancer tissues, hence, large efforts have been devoted to develop approaches to trigger the unloading of the drugs. Progress along these lines has been made over the last decade by the construction of liposomes that are sensitive to secretory phospholipase A2 (sPLA2), an enzyme that is upregulated in various cancer tissues. This progress has now lead to a new generation of liposomes, called Liplasomes, that have shown promising results in vivo for encapsulations involving, for e.g., doxorubicin and cisplatin.
The release mechanism involving sPLA2 has at the same time opened up for the use of novel lipid prodrugs or double prodrugs that can form liposomal systems and can be turned into active lipid drugs at the target, without the necessity for liposome drug loading. To demonstrate the feasibility of this approach, we introduced recently a new generation of sPLA2-cleavable anticancer lipid-based prodrugs, using ether prodrugs with either chlorambucil or retinoic acids. The prodrugs were chemically stable, formed unilamellar liposomes, were cleavable by sPLA2, and showed anticancer cytotoxicity only in the presence of sPLA2 with IC50 of a few µM. The mixing of the prodrugs with sPLA2-cleavable phospholipids was found to enhance the properties of the liposomal formulation and to strengthen its targeting capacity. In addition, it furnishes some advantages compared to the use of the prodrug alone; for example (1) control of the properties and stability of the liposomes, (2) enhance the hydrolysis profile of the prodrug and (3) exploitation of the permeability enhancing effect at the target membrane of the hydrolysis products of lipids, i.e., free fatty acids and lysolipids.

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Potentiometric measurements by using zinc oxide nanorods/nanowires to characterize a single cell

The nanostructure of zinc oxide (ZnO) such as nanorods, nanowires and nanotubes has interesting nanosurfaces in addition to its bulk properties. One of the properties is that these nanostructures are very suitable for intracellular measurements of pH, metal ions, glucose and also for cholesterol using potentiometric measurements. To adjust the sensor for intracellular measurements, the ZnO nanorods were grown on the tip of a borosilicate glass capillary (0.7µm in diameter) and functionalized with polymeric membrane or enzymes for intracellular selective metal ion sensors. Functionalized ZnO nanorods/nanowires show high sensitivity and selectivity. In addition to this the optical bulk properties makes zinc oxide wires suitable for photodynamic cancer therapy. The advantages are high spatial resolutions. The fabrication of such type of device aims to explain the methodology of ions sensing using functionalized ZnO nanorods and for photodynamic cancer therapy. This nanoelectrode device paves the way to enable analytical measurements in single living cells and treatment for cancer cell.

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LOCALIZED SURFACE PLASMON RESONANCE (LSPR) TRANSDUCERS FOR SENSING PROTEIN-CARBOHYDRATE INTERACTIONS

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In recent years, noble metal nanostructures that support localized surface plasmons (SPs) have been widely applied to sensing interactions in chemical and biological systems. Changes in the local refractive index in the vicinity of the nanostructures affect the intensity and position of the SP extinction band, thus making localized surface plasmon resonance (LSPR) spectroscopy a convenient tool for the study of biological interactions and for biosensing [1].
The specific interactions between carbohydrates and proteins play a predominant role in a variety of fundamental biological processes, such as cell-to-cell adhesion and communication, host-pathogen interactions, fertility and development, and cancer. Study of such interactions is therefore crucial for understanding of basic biological processes, as well as for the development of biosensors, primarily for diagnostics and drug development.
In the present work, LSPR transducers based on gold island films evaporated on glass and annealed were optimized for monitoring the specific interaction between Concanavalin A (Con A) from Canavalia Ensiformis and D-(+)-mannose. We synthesized a linker molecule composed of a terminal SH group, an undecanoic acid segment, and a 6-elements poly-ethylene glycol chain covalently bound to mannose or galactose, in order to form carbohydrate self-assembled monolayers (SAMs) on the gold islands. Specific response to binding of Con A to the mannose-modified (but not to the galactose-modified) LSPR transducer was demonstrated. Ellipsometry and FTIR measurements provided independent evidences for the specific recognition. Scanning electron microscopy was used to directly visualize the protein bound to the Au island transducer. A systematic study of the correlation between the Au island morphology and the transducer response enabled optimization of the sensing assay. The binding rate constants of the interaction between Con A and mannose were determined by performing kinetic experiments in a flow-cell system. A method for enhancing the LSPR transducers response was developed, based on gold nanoparticles modified with carbohydrates which bind specifically to the lectin immobilized on the transducer.
The results indicate that carbohydrate-modified gold island films can be used as convenient, effective and sensitive LSPR transducers for the study of carbohydrate-protein interactions. Biosensing application of this system to sugars relevant to pathogenic viruses, parasites or bacteria is underway.

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Cellular uptake and intracellular localization of siRNA-loaded DEAPA-PVA-g-PLGA-nanoparticles

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Biodegradable branched polyesters of the general structure poly[vinyl-3-(dialkylamino)alkylcarbamate-co-vinyl acetate-co-vinyl alcohol]-graft-poly(D,L-lactide-co-glycolide) have been synthesized and characterized with regard to the composition, molecular weight and degradation under physiological conditions [1]. Increase of amine substitution led to a reduction of the degradation time. For drug complexation, an increase could be observed with increasing amine substitution, whereas an increase of side chain length led to a reduction of drug complexation [2]. Degradation behavior, transfection properties, low cytotoxicity and a high knockdown efficiency with low amounts of siRNA used [3, 4] turn this polymer into an interesting and promising candidate for gene delivery.

The polymer was used for the preparation of siRNA-loaded nanoparticles. These particles were investigated with regard to the cellular uptake route and localization inside cancer cells. Therefore, fluorescent-labeled siRNA was used.

Nanoparticle preparation was performed by solvent displacement. The hydrodynamic diameters were determined by DLS. The dependance of particle uptake into H1299-EGFP cells on the incubation time and the temperature was studied by flow cytometry. Inhibition experiments focussed on clathrin- and caveolae-mediated uptake or uptake by macropinocytosis were performed. The intracellular localization was investigated by confocal laser scanning microscopy. A focus was put on the possible localization in lysosomes.

Nanoparticles with diameters of 100-150nm could be prepared successfully. In contrast to PEI-polyplexes, nanoparticle uptake could be observed to a high extent already after 30min and was complete during the first 2h. The uptake of the polyplexes increased permanently over a period of 4h. A decrease of particle uptake at 4 °C in comparison to 37 °C showed the uptake process to be of the active type. The inhibition of different uptake routes indicated that a clathrin-mediated uptake was the principal one. The siRNA of the nanoparticles could be clearly localized inside the cytosol. The distribution in the cytosol indicated a localization and trafficking of the particles in cellular compartments.
In conclusion, a high cellular uptake could be demonstrated for the siRNA-loaded nanoparticles. It is based on an active and predominantly clathrin-mediated process. Indications for particle localization in endosomes/ lysosomes were seen.

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See the abstract entitled "Ultrasounds-induced release from non-temperature sensitive paramagnetic liposomes for innovative MRI-guided drug delivery protocols." (first author Pierangela Giustetto)

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"Influence of surface modification on the intracellular uptake of SPIONs in tumor cells in vitro"

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To gain insight how nanoparticles (NPs) interact with cells it is necessary to have well characterised nanomaterials with defined properties available. In addition also standardised methods in cell biology are needed to study the effects caused by the interaction of the cell membrane with the NPs. In this study several surface modifications on one type of iron oxide core material were tested with regard to the intracellular localisation and the quantitative uptake of the different modified NPs by murine and human carcinoma cell lines.

Superparamagnetic FeOx-nanoparticles (SPIONs) with an iron oxide core of 15 nm and a silica shell of 5 nm were modified by coupling the respective ethoxy- or rather methoxysilane to the free OH-groups of the surface. The tested modifications were PEG-, amino-, PEG+amino-, carboxylic acid- and mercapto-silanes.

The motivation for choosing these modifications was to improve the dispersibility of the NPs in biological media and to investigate the influence of the functional groups on the intracellular uptake. NPs were tested in vitro using the C3H RS1 cell line, which was derived from a spontaneous murine mamma carcinoma. For comparison between animal and human cells the human mamma carcinoma cell line BT20 was chosen. After 24 h pre-culture the NPs were added to the cells with a concentration of 50 µg Fe/ml. Samples were taken in triplicates after 3, 6, 24, 30 and 48 h. The cell number was determined and the intracellular iron content was quantified with a colorimetric assay (Spectroquant®, Merck). Furthermore, to proof if the NPs are taken up and how they are compartmentalized, cells were analyzed after incubation with NPs by transmission electron microscopy (TEM).

The TEM pictures show that the NPs are taken up by the cells in high amounts. The NPs always appear in agglomerates in vesicular structures within the cytosol. The compartmentation seems to be independent from the respective surface modification. However, the quantification of the intracellular iron content shows differences in the intracellular uptake of NPs by the cells. Besides other factors like the protein corona or the agglomeration of the NPs these effect can be caused by different ζ-potentials and the resulting electrostatic interaction with the cell membrane.

To understand the underlying biological mechanisms, experiments with fluorescent NPs and simultaneous inhibition of selected uptake pathways will be performed in the near future.

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Polymeric micelles based on a phospholipid /polyaspartamidic copolymer for beclomethasone dipropionate delivery to the lungs
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Nanotechnological approaches have considerable potential for the treatment of pneumological diseases due to the opportunity of nanocarriers to control the temporal as well as the spatial distribution of drugs within the lung. Consequently, in the last few years new drug delivery devices intended to deliver drugs into the lungs have been widely developed.
In this work a novel drug delivery system for beclomethasone dipropionate (BDP) have been constructed through self-assembly of a pegylated phospholipid-polyaminoacid conjugate. This copolymer was obtained by chemical reaction of α,β-poly(N-2-hydroxyethyl)-DL-aspartamide (PHEA) with 1,2-Distearoyl-sn-Glycero-3-Phosphoethanolamine-N-[Amino(PolyethyleneGlycol)2000] (DSPE-PEG2000-NH2). Benefiting from the amphiphilic structure with the hydrophilic shell based on PHEA and PEG and many hydrophobic stearoyl tails, PHEA-PEG2000-DSPE copolymer was able to self assemble into micelles in aqueous media above a concentration of 1.23·10-7M, determined by fluorescence studies. During the self-assembling process in aqueous solution, these structures were able to incorporate BDP, with a Drug Loading (DL) equal to 3.0 wt %. A deep physicochemical characterization of empty and BDP-loaded micelles was carried out, including the evaluation of mean size, PDI, ζ potential, morphology and storage stability. Moreover, the excellent biocompatibility of both empty and drug-loaded systems was evaluated either on human bronchial epithelium (16HBE) or on red blood cells. The cellular uptake of BDP, free or blended into PHEA-PEG2000-DSPE micelles, was also evaluated, evidencing a high drug internalization when entrapped into these nanocarriers and demonstrating their potential for delivering hydrophobic drugs, after aerosolisation, in the treatment of pulmonary diseases like asthma and chronic obstructive disease.

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Elasticity and topography of endothelium cells in inflammatory response to TNF-A; an approach using atomic force microscopy methods

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The aim of presented study was to determine whether inflammation of endothelium evoked by inflammatory cytokine TNF-a; is related to changes in elasticity and structure of surface of endothelium cells. In order to answer the question, we used atomic force microscopy (AFM) to analyze the variation of elasticity and topography of surface of unfixed endothelium cells, line EAhy960, incubated in DMEM solution, stimulated with TNF-a; (1, 3, 10 ng/ml). Changes in cell morphology, surface of membrane and Young's modulus were examined in relation to concentration of TNF-a; and period of stimulation. The acquired data was compared to control conditions. TNF-a; converts the shape of endothelium cells in a concentration-dependent way, and has significant impact on the structure of cell membrane; cellular processes emerge, whose constraints depend strongly on duration of stimulation with TNF-a;. The changes in Young's modulus value are also strongly correlated with the concentration and the stimulation period. Initially, for short periods (1h, 3h) an increase in Young's modulus is observed. Then, for longer periods (more than 6h) its value decreases. Although biochemical mechanisms of the registered modulations are yet to be explained, one can advance a thesis that TNF-a; evokes a two-stage inflammatory response of endothelium tissue.
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Development and characterization of chitosan- sodium deoxycholate nanoparticles: a potencial gene delivery system for oral vaccination

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Abstract purpose: Chitosan is one of the most promising polymers for drug delivery through the mucosal routes because of its nontoxic, polycationic, biocompatible, and biodegradable nature, and particularly due to its mucoadhesive and permeation-enhancing properties. Bile salts are known to interact with lipid membranes, increasing their permeability. So, the addition of bile salts to chitosan matrices may improve the delivery characteristics of the system, making it suitable for mucosal administration of bioactive substances. In the present study we have developed chitosan-sodium deoxycholate (CS/DS) nanoparticles and evaluate their potential as gene delivery carriers. The physicochemical properties of the particles were analyzed as well as transfection studies in gastric cell cultures, which may be employed in future in vivo studies. Methods: Nanoparticles of chitosan and sodium deoxycholate were obtained via a mild ionotropic gelation procedure using different weight ratios and were used to encapsulate plasmid DNA expressing a “humanized” secreted Gaussia Luciferase as reporter gene (pGLuc, 5.7 kDa). Mean particle size, polydispersion index and zeta potential were evaluated in order to choose the best formulation for posteriors in vitro studies. Transfection efficiency of CS/DS-pDNA nanoparticles into AGS and N87 gastric carcinoma cell lines (moderately and well differentiated adenocarcinoma, respectively) was determined by measuring the activity of expression luciferase. The end-point used on the cytotoxicity assay was the MTT reduction. Results: The average size range from 403-153 nm and a positive zeta potential ranging from +46.2 to +56.9 mV was showed for nanoparticles produced changing the proportion between Chitosan : Sodium deoxycholate weight (w:w) from 1:0.25 to 1:1.65. Independently of the amount, all the pDNA was efficiently encapsulated into the NP. CS/DS nanoparticles containing encapsulated pDNA were able to transfect both AGS and N87 cell lines, being more effective with AGS cells, the less differentiated cell line. The highest enzymatic activity was achieved with 20% pDNA encapsulated and after 24h of transfection time. No evidence of cytotoxicity was observed for the CS/DS nanoparticles neither with or without pDNA. Conclusions: Chitosan-sodium deoxycholate nanoparticles carrying pDNA were successfully produced and due to the high levels of transfection efficiency they could be a new potential vehicle for oral delivery of pDNA.

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Membrane potential depolarization softens vascular endothelial cells
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The mechanical stiffness of endothelial cells (cells that form the inner layer of all blood vessels) determines vascular function for it influences their production of the vasodilator nitric oxide (NO). Endothelial cells are constantly exposed to mechanical forces (shear stress) exerted by the blood flow that dynamically deform the cells. This deformation can activate the endothelial nitric oxide synthase. When endothelial cells stiffen, they become less deformable and, as a consequence, NO production is reduced. This results in a more contracted state of the vascular smooth muscle cells, an increased peripheral resistance and high blood pressure. However, despite the importance of mechanical stiffness for endothelial performance, the regulation of cell stiffness is not yet understood. There is evidence that a possible regulator could be the electrical plasma membrane potential difference. To test this hypothesis, we directly related changes in the endothelial membrane potential to changes in cellular mechanical stiffness. To this end, the electrical potential was manipulated in different ways and the induced potential changes were then simultaneously measured with changes in cell stiffness. Such combined measurements in single cells were made possible by a novel approach that was recently developed in our laboratory. This nanotechnique involves a single setup of an atomic force microscope (AFM) attached to an inverted fluorescence microscope and combines AFM-based nano-indentation stiffness measurements with fluorescence (bis-oxonol)-based membrane potential measurements. The present study shows that membrane depolarization is associated with softening of endothelial cells. Three different depolarization protocols were applied all of which led to a similar and significant decrease in cell stiffness (about 20 %). Moreover, it is demonstrated that it is rather the membrane potential depolarization and not cell swelling that causes the stiffness decrease of the cells under all three depolarizing conditions. A model is proposed according to which a change of the electrical field across the plasma membrane can be sensed by the submembranous actin network, regulating the actin polymerization/depolymerization ratio and thus cellular mechanical stiffness. Depolarization-induced endothelial cell softening could play a role in flow-mediated, nitric oxide-dependent vasodilation.

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Cell labelling based on targeting exofacial protein thiol with GdIII complexes.
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The extracellular side of the plasma membrane of many mammalian cells display as many as 10E9-10E10 protein thiol groups per single cell, the exact number being dependent upon the redox conditions of the extracellular milieu. Exofacial Protein Thiols (EPTs) are at a maximum in reducing conditions (such as hypoxya), whereas they are partially transformed into disulfide, S-thiolated, S-nitroso, or sulphenic forms under oxidizing conditions. Then, Exofacial Protein Thiols (EPTs) can be thought of as a target for imaging procedures aimed at visualizing the redox state of the extracellular microenvironment, which may be in turn indicative of patho-physiological alterations. EPTs in the reduced form are quite reactive and can be chemically labeled by a suitably designed MRI contrast agent (compound GdL1A), composed of a GdDO3A based structure, a 2-pyridyldithio function for the recognition of EPTs and a flexible spacer connecting them.1,2 Compound GdL1A can react with cell EPTs to form a disulfide bridged adduct, followed by the internalization of the GdDO3A complex. In vitro labelling experiments with human myeloid leukemia K562 cells and murine melanoma B16 cells showed high levels of EPTs-mediated gadolinium uptake. In vivo labeling of tumor cells in mice grafted with B16 cells has also been demonstrated. As the levels of EPTs are correlated with the extracellular redox microenvironment, the proposed Gd(III) probes can be thought as redox responsive agents. Nanosized Gd-based MRI probes containing disulfide bonds, whose MRI signal enhancement is responsive to the redox microenvironment, will be finally presented.

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Effective encapsulation, protease and temperature protection of proteins using polymersome

Therapeutic biomolecules such as proteins present major issues associated with their delivery method to targeted sites because of the presence of particular enzymes (a.k.a. Protease) in biological fluids which cause their degradation. For instance, endogenous clotting cascade enzymes released by damaged cells or exogenous enzymes from contaminating bacteria can contribute to the inactivation of proteins. Temperatures higher than 37°C during transport or storage of proteins in solution often lead to the unfolding of the native structure inactivating the proteins.

In this study we encapsulated a model protein in PMPC25PDPA70 polymersomes in order to investigate the protective effect of a confined environment with controlled dimensions from temperature and enzymatic degradation of proteins. Myoglobin, a globular protein of 153 amino acids folding around a central HEME prosthetic group, has been selected as a model protein because its degradation can be easily monitored with UV-Vis spectroscopy by following the shift and reduction of the characteristic absorption band at 409nm (Soret band). It contains eight alpha helices and a hydrophobic core and has the specific function of delivering oxygen to muscle tissues.

We challenge the stability of myoglobin by adding small protease, namely trypsin, to the loaded polymersomes dispersions or by increasing the temperature with a programmed ramp monitoring the relative absorption spectra over time. The effect of sonication and extrusion of the protein-polymersome mixture on the encapsulation efficiency was also analyzed. The fraction of protein loaded polymersomes was purified by GPC using Sepharose 4B. DLS analysis was performed to characterize the ability of the polymer to self assemble into colloidally stable vesicles at neutral pH in the presence of the protein.

The results show that the encapsulation efficiency is affected by the sonication time of the polymersome-protein solution, whilst the extrusion merely uniforms the size of the nanoparticles according with DLS results.

The protease assay after incubating polymersomes in 10µg/mL trypsin at 37°C, for 1 and 24 hours, showed that the Soret band was stable compared to the free myoglobin. The temperature degradation assay obtained by monitoring the UV-Vis spectra from 30 to 70 degrees and CD spectra from 20 to 95 degree, showed in both cases the stability of the loaded protein compared to the control sample.

These results confirm that polymersome are a good candidate for protein encapsulation and stabilization and it will be used as delivery system for therapeutic applications.

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Hira, a putative histone chaperone of differentiation and early development.
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Hira is a putative chaperone for the replacement histone H3.3 and, in some species, a transcriptional co-regulator. Previous work has identified a general role for Hira in early development, with both chick neural crest knockdown experiments and constitutive knock out in the mouse indicating Hira is required for normal development. But how Hira acts during these processes has not yet been elucidated. At the molecular level, Hira is associated with H3.3 deposition within chromatin in specific regions of genes. We show that Hira is not required for maintenance of a stem-cell like state, but is required during differentiation processes. Interestingly, the ability of embryonic stem cells to renew is linked to the ability of cells to remain in a proliferative state, and undifferentiated embryonic stem cells spend most of their time in S phase, where the replication histone replacement machinery is active. Hira has been shown to be required only during replication-independent processes, which explains why we observe phenotype only in non replicative phase of the cell cycle. Contrary to general opinion, for the first time we demonstrate that Hira is not a random putative histone chaperone required in general mechanisms of non replicative processes, but in fact acts very specifically to disrupt specific pathways, while having no role in others. Moreover we correlate what is observed in our embryonic stem cell system to what is observed in the embryo, and propose a general mechanism for the role of Hira as the putative histone chaperone of early development in mammals. We also developed conditional mice, and confirm that Hira is acting in a tissue and time dependent manner. We then demonstrate in vitro and in vivo that Hira is actually a very tightly regulated putative histone chaperone that acts in specific, rather than general pathways in non replicative processes. We finally demonstrated that this action is actually regulated by post-translational modifications. Altogether, for the first time, we explain a role, and propose a model of how this pleiotropic protein is vital for life.

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Size dependent accumulation of nano- and microparticulate carriers in the inflamed intestinal tissue – a novel targeting strategy for the treatment of inflammatory bowel diseases

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In spite of colon targeted dosage forms, anti-inflammatory therapy in inflammatory bowel disease (IBD) is often severely hindered by diarrhea which shortens drug carrier residence time at the site of inflammation. Passive targeting of drug carriers to the inflamed intestinal mucosa has potential to improve IBD therapy. Previously, a specific accumulation of nanoparticles of ~100 nm size in inflamed areas of the colonic mucosa was demonstrated in a rat colitis model while no such effect could be observed for microparticles of 1 - 10 µm size [1]. In agreement with these findings poly(L-lactide-co-glycolide) (PLGA) nanoparticles loaded with the anti-inflammatory drug rolipram showed increased therapeutic activity and reduced central nervous adverse effects in the rat model [2]. To take the next step from the animal model to a clinical evaluation of the new therapeutic concept in humans, a further exploration of this size dependent accumulation is needed both in-vitro to better understand the mechanism and in-vivo in IBD patients.
PLGA was covalently labeled with fluoresceinamin and nanoparticles (~330 nm) and microparticles (~3.5 µm) were prepared by emulsion solvent evaporation technique. Clinical investigations in human IBD patients were approved by the local ethics committee, and informed written consent was obtained from each patient. 5 control patients and 25 patients with IBD (10 patients with Crohn´s disease, 15 patients with ulcerative colitis) were investigated. 10 patients received nanoparticles and 15 patients received microparticles. A 30 ml 0.9% NaCl enema containing 1x10^9 microparticles or 1x10^13 nanoparticles, respectively was applied to the patients. After 2 h confocal laser endoscopy was performed to visualize nanoparticles in areas of different stages of inflammation. For in vitro investigations, Caco-2 cells, grown to confluency and stimulated with IL-1β 10 ng/ml for 48 h to induce inflammation, were incubated with nano-or microparticles suspensed in cell culture medium for 2 h. Afterwards, cells were washed three times with PBS, fixed and the particle allocation in the model was analyzed using confocal laser scanning microscopy.
Indeed, particles showed a significantly increased affinity towards mucosa with epithelial defects (erosions and ulcerations). While the in-vivo study so far revealed the most pronounced effects for microparticles, with a strong correlation being observed between endoscopic activity score and extent of particle accumulation, the in-vitro model demonstrated enhanced adherence of nanoparticulate carriers. In conclusion, the in vivo data support the potential of drug containing carriers to target IBD lesions with the size accumulation relationship needing further investigation.

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Effect of Nanoscale Collagen Arrangement in Gel Substrates on Corneal Stem Cell Differentiation

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Purpose
Clinical expansion of corneal stem cells typically requires the use of amniotic membrane (AM) as a biomaterial. However, AM is not usually characterised before clinical use and previous studies have found significant variation in structural and chemical composition. Furthermore it lacks the scalability of an engineered polymer construct. In order to improve the surgical outcome of ocular surface reconstruction, we investigated the best preparative condition for AM when used as a stem cell substrate and whether plastically compressed collagen scaffold can effectively replace AM as a corneal stem cell substrate.

Materials and Methods
Corneal stem cells were isolated from bovine limbus and expanded upon either AM (intact or denuded) or collagen type I gels with a tractable nanoscale arrangement(uncompressed or compressed). Using supplemented media, cell culture continued for 2 weeks followed by 1 week under â€˜air-liftingâ€™ conditions. The growth and differentiation of limbal epithelial cells was quantified by microscopy, Western blotting and QPCR.

Results
Limbal epithelial cells expanded to form 4-6 layers on both intact and denuded AM. On denuded AM the proportion of undifferentiated cells remained unaltered following air-lifting. However, when using intact AM the number of differentiated cells increased significantly following air-lifting. Limbal epithelial cells were successfully expanded upon both uncompressed and compressed collagen gels resulting in corneal constructs with similar levels of cell stratification and gene/protein expression profiles for common makers against un-differentiated (K14) and differentiated (K3/12) corneal epithelia. However, only those cells expanded upon the compressed gel where shown to contain normal cell surface attachment structures.

Conclusions
These results have important implications for both basic and clinical research. From a tissue engineering perspective, this study demonstrates that the phenotype of expanded limbal epithelial cells is influenced by the nanoscale arrangement of the collagen substrate. Compression of the collagen gel creates a stiffer substrate, promotes cell adhesion whilst intact AM promotes differentiation following air-lifting. Furthermore, compressed collagen gel can adequately support limbal epithelial cell expansion, stratification and differentiation to a degree that is comparable to the leading conventional scaffold, AM.

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TITLE:
Interaction of liposomes with proteins measured by Surface Plasmon Resonance correlates with their in vivo circulation time.

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INTRODUCTION:
An important process influencing the circulation time of liposomes and other nanoparticles is opsonization by blood proteins. Proteins adsorbed to the outer shell of liposomes are recognized by macrophages of the reticuloendothelial system (RES) triggering their clearance from the circulation. Incorporation of polyethylene glycol (PEG) conjugated lipids into the lipid bilayer decreases the blood clearance of liposomes considerably. It has been hypothesized that the PEG chains create a 'steric barrier' which prevents protein adsorption to the liposomal surface.
A direct relation between the amount of adsorbed protein and the circulation time has previously been established. This relation could be useful in the screening of (new) liposomal formulations for their circulation time in vivo. However, quantification of adsorbed proteins in a series of liposomal formulations is time-consuming, and typically involves washing steps which could lead to underestimated results.
Surface plasmon resonance (SPR) is a powerful tool for real-time monitoring and measuring interactions between different molecules. This study aims to explore the potential of SPR to quantify the interactions of PEGylated liposomes with proteins and to correlate this with their in vivo circulation time.

EXPERIMENTAL METHODS:
Surface plasmon resonance (SPR) (Biacore 3000, GE Healthcare) was used to evaluate the interaction of (non-)PEGylated liposomes with several physically relevant proteins. DPPC Liposomes were grafted with either DPPE-PEG2000, at a molar grafting density of 2.5%, 5.0% or 7.0%; or DPPE-PEG5000, at densities of 0.5%, 1.5% or 2.5%.
The proteins that were immobilized on a CM5 sensor chip using amine coupling included human serum albumin, α2-macroglobulin, β2-glycoprotein and fibronectin.
Liposomal formulations were injected at a flow rate of 5 μL/min for 40 minutes while monitoring the change in plasmon resonance angle. To correlate the protein interaction of these liposomes with their longevity in vivo, radiolabelled ([3H]DPPC) liposomal formulations with the same compositions were injected in NMRI mice. Blood samples were taken 15, 30, 60, 120, 240 and 360 minutes after injection and analysed using liquid scintillation counting.

RESULTS AND DISCUSSION:
Grafting density of PEG onto liposomes was inversely correlated with the protein interaction measured with surface plasmon resonance; higher grafting densities of either PEG2000 or PEG5000 showed less interaction with all proteins. This finding was consistent with the circulation lifetime of each liposomal formulation, where higher grafting densities of either PEG2000 or PEG5000 showed longer circulation times.
These results show that SPR can be used for characterization of liposomal formulations, in particular their ability to prevent the adsorption of proteins. As there is a direct relation between the extend of

protein adsorption and the in vivo longevity of a particle, SPR can be used as a screening tool in the optimalization of the circulation time of liposomes and other particles.

CONCLUSION:
Surface plasmon resonance can be considered as an interesting and promising tool for the evaluation of liposome-protein interactions in vitro, as well as for predicting the circulation time of liposomal formulations in vivo.

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The impact of ultrasound on liposomal drug release
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Successful cancer therapy requires that the therapeutic agents reach the tumour cells and inactivate them, whereas damage of normal tissue should be minimized. Encapsulated drug has improved tumour to normal tissue uptake compared to free drug. However, distribution of the drugs as well as the capsules are heterogeneously distributed in the tumour tissue. Ultrasound may overcome this by improving drug delivery in various ways i.e. enhancing drug release, improving transcapillary transport and transport through extracellular matrix, as well as across plasma membrane. These various steps require different optimal ultrasound exposures. Therefore a challenge is to determine the optimal frequency, acoustic pressure or intensity, duration, and duty cycle (determined by the pulse length and pulse repetition frequency (PRF)). The exact mechanism for ultrasound mediated release is poorly understood, but cavitation may be an important factor. In this work we aim to investigate the ultrasound exposure parameters that maximize drug release from liposomes in solution and in collagen gels used as a model for extracellular matrix. Two ultrasound transducers (300 kHz and 1 MHz) were employed. The release of fluorescent calcein from liposomes in solution was measured as the increase in fluorescence intensity using a spectrophotometer. In collagen gels the release and distribution of calcein were studied using confocal laser scanning microscopy and the fibrillar collagen network visualized using the second harmonic optical signal. Drug release was most efficient at 300 kHz compared to 1 MHz. A certain threshold in acoustic pressure had to be overcome to obtain drug release, and the pressure needed was lower at lower frequencies. The release was found to increase with exposure time, and followed a first order kinetics. Above the threshold the release increased with increasing acoustic pressure or intensity, and increased linearly with mechanical index (MI) which depends on the ultrasound pressure and frequency. This indicates that the release of the drug from liposomes is caused by mechanical rather than thermal effects. Thus, cavitation either inducing liposomal collapse or sonoporation may be the mechanism for the increased release. The results demonstrate that ultrasound has a great potential in enhancing drug release from liposomes thereby improving cancer therapy.

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Biodistribution of PEGylated SPIONs in a tumor bearing mouse model

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Superparamagnetic iron oxide nanoparticles (SPION) have become important for numerous biomedical applications, such as magnetic resonance imaging, drug delivery, tissue repair and Nano-Cancer® therapy in recent years.
Nano-Cancer® therapy is a technique based on AC magnetic field-induced excitation of biocompatible SPIONs for selective tissue heating. In current practice the SPIONs are directly injected into the target region where they generate heat by Brownian and Néel relaxation processes in an externally applied alternating magnetic field [1]. Pre-clinical and clinical data have demonstrated efficacy of this approach [2].
To administrate SPIONs intravenously (i.v.) two conditions are essential. First of all, the suspension has to be stable against agglomeration under physiological conditions. The second requirement is immunological stealthiness to avoid nanoparticle (NP) elimination from the blood circulation by the immune system, which leads to prolonged circulation times. These properties can be modulated by coating of the particles with polymers, such as polyethylene glycol (PEG).
During tumour growth an extensive angiogenesis with defective endothelial cells takes place. This enables macromolecules and NPs to extravasate into the tumour. Due to the impaired lymphatic drainage the macromolecules and NPs remain in the tumour for an extended period [3]. This is called the enhanced permeability and retention (EPR) effect. Our hypothesis is that this effect, in combination with the prolonged circulation times of the PEGylated NPs, will lead to a passive accumulation of the NPs in solid tumours. Furthermore we investigated if the EPR effect can be improved by locoregional heating of the tumour.
In order to determine the biodistribution of PEGylated SPIONs and to figure out if the accumulation in the tumour is high enough for Nano-Cancer® therapy, an isogenic in vivo model was used. SPIONs with a Fe_2O_3 core of 15 nm and a SiO_2 shell of 5 nm were modified by coupling the PEG-groups as an methoxysilane to the free OH-groups of the surface. These particles were injected i.v. into the tail veins of mammary carcinoma bearing C3H mice. At various time points the mice were sacrificed and the organs were extracted for qualitative (microscopy) and quantitative (colorimetric assay) determination of NP distribution.
The obtained results demonstrate that the biodistribution of PEGylated SPIONs altered time-dependently and that the amount of PEGylated SPIONs varied in different organs. Additionally it was pointed out, that they accumulate passively in the tumor due to the EPR effect, which can be modified by locoregional heating.

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Formulation Development of Biodegradable Nanovectors for Anthracycline Delivery
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ABSTRACT
BACKGROUND:
Doxorubicin (DOX) is an anthracycline topoisomerase inhibitor. It is a highly potent antineoplastic agent used against broad spectrum of malignancies. However its therapeutic efficacy is limited due to dose dependent cardiotoxicity and myelosuppression. In an effort to improve its antitumor efficacy and to reduce the toxicity various drug delivery systems have been developed. In one of such approaches, nanoparticles (NP) could be engineered to target the drug to tumor site and remain in the circulation for longer time because of their nano scale size. Also, it is a promising way to reduce adverse drug effects while maintaing the therapeutic benefit.
PURPOSE:
The aim of the present investigation was to formulate nanovectors of DOX for controlling the drug release, extending the blood circulation and therby improved therapeutic benefit.
METHOD:
Biodegradable poly (lactic-co-glycolic acid) (PLGA) NP's were prepared by double emulsion method (W1/O/W2) using dichloromethane (DCM) as an organic solvent. Effect of different surfactants like Poly (vinyl alcohol) (PVA) and Tween 80 and their concentration on particle size, polydispersity index (PDI), zeta potential (ZP) and entrapment efficiency (EE) and In vitro drug release studies were then evaluated. The structure and morphology of NP's were characterised by FT-IR, DSC and SEM studies.
RESULTS :
DSC studies revealed amorphous nature of the NP's. It was found that type and concentration of the surfactant could significantly affect the NP formation and its properties. The optimal formulations with 1% PVA and 2% Tween 80 were developed. The particle size found to be 193 and 137 nm respectively. The PDI and ZP were found to be 0.131 and -15.4 mV for PVA-NP's and 0.417 and 25.8 mV for Tween 80-NP's. The %EE of NP's was as high as 21.87%. Initial burst release followed by sustained release observed during drug release studies.
CONCLUSION:
The data indicates potential of entraping this anthracycline (DOX) in nanoparticles, as well as better vectors to specify the drug to tumor cells and reduce adverse drug events while sustaining the therapeutic efficacy.
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Curcumin entrapped into lipid nanosystems improves inhibition of neuroblastoma cancer cell growth activating Hsp70 protein.

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Curcumin is a natural anti-cancer compound utilized on a wide variety of human cancer cell lines and animal carcinogenesis models. However, its clinical application has been limited for its minimal systemic bioavailability. Nanoparticle-based drug delivery approaches have the potential for rendering hydrophobic molecules such as curcumin dispersible in aqueous media, thus overtaking the limits of its poor solubility. Here, we reported the preparation and chemical-physical characterization of Nanostructured Lipid Carriers (NLC) containing curcumin, based on Imwitor, Compritol Precirol as lipid matrix. By in vitro experiments, we have demonstrated that these nano-systems are able to carry curcumin into LAN5 neuroblastoma cells and their effect on cell mortality is higher than free curcumin. However, the best results were obtained when the NLC-c system was utilized. Moreover, we have demonstrated that curcumin activates Hsp70 protein and that this effect is enhanced when the same dose of curcumin is administered as drug-loaded NLC. The obtained results clearly suggest that these nanoparticles are a potential curcumin delivery systems and encourage, in future, for planning in vivo studies towards cancerand other diseases that might benefit from the curcumin effects.

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Polyglutamates with Novel Architectures, Richard M. England and Maria J. Vicent, Polymer therapeutics laboratory, Chemical biology department, Centro de Investigacion Principe Felipe, Valencia, Spain

Star polymers have interesting solution and solid state properties resulting from their three dimensional shape. Typically these properties are derived from differences in hydrodynamic volume and also the presence of additional chain end groups compared to linear polymers of similar composition. These differences also extend to differences in degradation pathways under physiological conditions[1] and also different cellular uptake rates in vitro.[2] With these factors in mind we have endeavoured to create star polymers composed of Poly(Glutamic Acid) whilst utilizing the Schlaad initiation method that helps to prevent side reactions that would otherwise lead to byproducts and subsequently broad molecular weight distributions.[3] These materials will be used for drug conjugation in the development of therapeutic agents and/or imaging systems in cancer research.

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IOPROMIDE AND Tc-99m LOADED NANO-SIZED TUMOR TARGETED LIPOSOMES FOR TUMOR IMAGING BY SPECT/CT

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With the developing technology and improvements in the computer engineering, science enable refinements and the generation of novel imaging modalities that combine imaging instruments that are used in Nuclear Medicine provided metabolic and Radiology provided anatomical information. Depending on these developments, there is an essential need for the novel contrast agents that specifically delivers to the target, gives sufficient signal/contrast intensity and less target/background contrast ratio for obtaining better images with less time consuming. Liposomes have been using for a long time to develop contrast agents for different imaging modalities because of their properties such as biocompatibility, bioavailability, loading capacity of different contrast agents and ligands in the aqueous core and/or on the lipid bilayer.

When visualization of small pathological areas, such as tumors, is required, the local contrast agent concentration in these areas becomes crucial. PEGylated liposomes have been suggested to increase the local concentration and to prolong the plasma half-life of contrast agents. To increase the accumulation of drug-loaded liposomes (including liposomes loaded with contrast agents) in various targets including tumors, the use of specifically targeted liposomes was also suggested. For this purpose, plain or long-circulating liposomes are additionally modified with target-specific ligands, usually monoclonal components against characteristic component of the pathological tissue (1,2). Earlier, family of natural antinuclear autoantibodies with nucleosome(NS)-restricted specificity capable of specific recognition of a broad variety of live cancer cells via cancer cell surface-bound NS, which are released by apoptotically dying neighboring cancer cells were identified (3).

Here, we demonstrate that Iopromide and Tc-99m loaded liposomes can be additionally modified with the monoclonal anticancer antibody 2C5 (mAb 2C5) possessing the nucleosome-restricted specificity via the PEG spacer.

Liposome-bound antibody immunoliposomes specifically recognize various cancer cells in vitro and target an increased amount of contrast agent/radionuclide to their surface compared to antibody-free liposomes. The results showed that mAb 2C5 modified PEGylated liposomes containing Iopromide specifically bind to the cancer cells and binding capacity of immunoliposomes are 4-5 times more than control non-modified liposomes in vitro. The interaction of Iopromide containing PEG-Liposomes with cancer cells were visualized by fluorescent microscopy

As conclusion, Iopromide and Tc-99m loaded cancer cell targeted immunoliposomes may represent promising agents for enhanced tumor imaging by SPECT/CT.

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Nanomechanics of vascular endothelial cells studied with a hybrid fluorescence atomic force microscope

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Malfunction of blood pressure regulation is a dominant risk factor and plays an important role in arterial hypertension and cardiovascular disease. Nitric oxide (NO), produced by the endothelial NO synthase (eNOS), is the key player in the control of blood pressure. One of the major activators of eNOS is blood flow mediated shear stress. The cellular sensitivity to shear stress is influenced by the cell's deformability, known as mechanical stiffness. Soft cells produce NO whereas stiff cells fail. However, it is not known whether the nanomechanics regulate NO release or vice versa.

To address this question, it is necessary to simultaneously measure both nanomechanics and NO release. Using a combined setup of an atomic force microscope integrated into a fluorescence microscope we analyzed stiffness of single living endothelial cells by nano-indentation. eNOS activity was simultaneously measured by the application of a fluorescent NO indicator. Transient cell softening was induced by the acute application of aldosterone, a salt and water retaining a steroid hormone. As expected NO release and cell softening correlate with each other. When eNOS was inhibited by a specific blocker, NO release was abolished whereas aldosterone-induced changes in mechanical stiffness remained unaffected. Furthermore, an eNOS-independent NO donor intensively increased intracellular NO concentration while cell stiffness remained constant.

Taken together, the simultaneous measurement of NO-related fluorescence and mechanical stiffness in one and the same living cell gives a clear answer: NO does follow nanomechanical alterations in vascular endothelium and not vice versa.

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Novel antimicrobial polymer conjugates designed to treat Gram-negative infections and circumvent toxicity and resistance.

Bacterial resistance to antibiotic therapy is a major world health problem [1]. Decreases in the design/development of new antibiotic entities have been mirrored by increasing resistance to currently available antibiotics [2]. Consequently, clinicians are increasingly turning to older antimicrobials which are effective against Gram-negative organisms but have toxicity issues (e.g. polymyxins) [3]. The effective utilization of these agents is therefore severely limited in practice. A safe, effective delivery system for these antibiotics would revolutionize the treatment of patients with these infections.

Given that polymer therapeutics have demonstrated significant improvements in protein and peptide delivery [4], we have developed the first bioresponsive polymer conjugate to target the delivery of antimicrobials to sites of inflammation; increasing bioactivity/ bioavailability, decreasing systemic toxicity and improving clinical effectiveness. As a proto-typical model, dextrin conjugates of colistin (1,400 g/mol) have been developed and are being characterized to optimise diffusion, delivery and clinical effectiveness. As such, we have recently shown that dextrin can be used as a partner polymer in the context of a new concept named 'Polymer masked-UnMasked Protein Therapy' (PUMPT) [5]. PUMPT relies on the ability of dextrin to mask colistin in transit (reducing neuro/nephrotoxicity), but after α-amylase-mediated degradation of dextrin, full enzyme activity can be restored.

A library of dextrin-colistin conjugates was synthesized using dextrins of low (~8,000 g/mol) and high (~50,000 g/mol) molecular weight (2-60 mol% succinoylation). The resulting conjugates were typically 10,000-22,000 g/mol and 55,000-77,000 g/mol, respectively, with a colistin content of 5-22% w/w (< 3 % free colistin). Conjugate unmasking by physiological concentrations of α-amylase was observed using GPC and FPLC and demonstrated optimum release kinetics from conjugates containing low molecular weight dextrin with 2.5 mol% succinoylation. When the antibacterial activity of the polymer-bound colistin towards Pseudomonas aeruginosa (PA01) was assessed using a minimum inhibitory concentration (MIC) assay, the antimicrobial activity was effectively masked (MIC = 512 μg/mL). However, when the dextrin-colistin conjugates were pre-incubated with physiological concentrations of α-amylase time-dependant re-instatement of antimicrobial activity was observed (up to 4-fold decrease in MIC value). The greatest reduction in MIC value was seen for conjugates containing minimally modified low molecular weight dextrin. Current experiments are characterizing the antimicrobial activity towards drug resistant Gram-negative bacteria.

These results demonstrate the potential of polymer conjugation as a novel means of improving the safety profile of otherwise toxic peptide antimicrobials and shows that this approach warrants further development.

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Immunity by formulation design: Customizing the adjuvant properties of cationic liposomes by rational combination with immunopotentiators

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Cationic liposomes are promising delivery vehicles for vaccine antigens and can in combination with immunopotentiators act as potent vaccine adjuvants. An adjuvant system (designated CAF01) based on cationic liposomes composed of dimethyldioctadecylammonium (DDA) and the immunopotentiator trehalose 6,6¡¯-dibehenate (TDB) is currently in phase I trials with the tuberculosis fusion antigen Ag85B-ESAT-6 due to its ability to induce a strong cell-mediated Th1 immune response as well as a humoral response. A weakness of many vaccine adjuvants including CAF01 is though the lack of induction of CD8+ T-cell responses against protein antigens, which are required for protection against challenging and difficult infectious diseases such as AIDS and for therapeutic cancer vaccination. Polyinosinic-polycytidylic acid (poly(I:C) is an immunostimulatory synthetic dsRNA molecule recognized by toll-like receptor 3 (TLR3) expressed by antigen-presenting cells. Stimulation of TLR3 leads to induction of type I interferons, which are linked to the ability to generate CD8+ T-cell responses. Immunization of mice with the model antigen ovalbumin and DDA/TDB liposomes combined with poly(I:C) via a carefully optimized preparation procedure induced strong CTL responses, indicated by the presence of large populations of antigen-specific CD8+ T-cells. In addition, antigen-specific CD8+ T-cell responses were also induced upon vaccination with DDA/TDB/poly(I:C) liposomes combined with proteins and peptides antigens from influenza, tuberculosis and HIV. Importantly, where administration of soluble poly(I:C) caused rapid production of TNF alpha and IL-6 in serum, administration of poly(I:C) in combination with DDA/TDB liposomes prevented this induction. As systemic production of pro-inflammatory cytokines is a known and undesirable adverse effect of poly(I:C), the co-delivery of poly(I:C) with DDA/TDB liposomes can thereby serve to remove one of the major obstacles in using strong CD8-inducng agents such as poly(I:C) in future subunit vaccines.

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NEW VEHICLES FOR DRUG DELIVERY: MACROPOROUS SOLID FOAMS PREPARED FROM HIGHLY CONCENTRATED EMULSIONS

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The polymerization in the continuous phase of highly concentrated emulsions allows the preparation of solid foams with very high pore volume that can be used as drug delivery systems. The objective of this research has been the incorporation of model lipophilic and hydrophilic drugs (ketoprofen and clindamycin hydrochloride respectively) to polystyrene macroporous hydrophobic solid foams, previously prepared by a method based on the PIT emulsification method. Drug release to a receptor solution was studied by a molecular dialysis method.

To ensure homogeneous impregnation and drug delivery from the macroporous materials, the monoliths obtained were immersed in water/alcohol solutions (1:1 w/w) containing 2 %, 5% or 10 % of ketoprofen or clindamycin hydrochloride, respectively. The solid foams were then dried at 50ºC for 24 h after the incorporation of the drug, which was quantified by the weight increase of the materials.

Diffusion experiments were carried out in dialysis bags containing the macroporous materials with the drug and filled with PBS (pH=7.4), which was also used as a receptor media, or in a dissolution test equipment. Both, dialysis bags and membranes for dissolution test, were porous cellulose membranes with a molecular weight much bigger than the molecular weight of ketoprofen and clindamycin. The diffusion cell consisted on a cylindrical thermojacketed glass vessel, thermostated at 37ºC. The diffusion rate of the drug dialysis bags into the receptor was determined by measuring the amount of drug diffused as a function of time as determined by UV espectrofotometry (233 nm for ketoprofen and 210 nm for clindamycin hydrochloride).

The polystyrene materials used in this work as potential drug delivery systems contain macropores with a certain polydispersity, preserving the structure of the continuous phase of the emulsion which served as template for polymerisation.

The impregnation is the critical point of the process, since it depends on the size and shape of the monolith as well as on its weight. It has been observed that the most homogeneous impregnation results are obtained by the immersion method of the solid foams in a solution with the drug and the latter drying.

Weight uptake by the monolith disks increases linearly with the concentration of drug in the aqueous solution from 23% to 67% and weight uptake in solutions containing 2% to 10% of drugs, respectively. However, the amount of active principle in the monolith does not always seem to be related to the drug release, and varies from 80,8 % to 93,4 % at 24 h.

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Ultrasounds-induced release from non temperature sensitive paramagnetic liposomes for innovative MRI-guided drug delivery protocols.

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Non, or minimally, invasive in vivo visualization of drug release triggered by externally-applied physical stimuli, like heating, light or ultrasounds (US) is an emerging topic in molecular medicine. As far as US is concerned, most of the published investigations were performed using nano- or micro-bubbles in which the presence of the gas-filled core makes cavitation (and US detection) possible. However, the use of imaging modalities with an improved spatial resolution and wider diagnostic/therapeutic potential like MRI would be very useful.

Liposomes, nanovesicles endowed with an aqueous core, are extensively used as drug delivery nanocarriers and are also under intense scrutiny in the field of MRI contrast agents where they represent one of the most promising nanoplatform for designing highly-sensitive probes.

The most straightforward approach to design liposomal MRI probes whose image contrast can report about the US-induced release of the nanocarrier payload is the encapsulation of a large amount (100-200 mM) of a paramagnetic Gd(III) agent in the vesicle. In fact, the ability of the encapsulated agent to generate a MRI (T1) contrast is strongly limited by the water permeability of the vesicle membrane, but upon the probe release, such a T1 “quenching” is removed and a considerable contrast enhancement can be observed.

In this contribution, we demonstrate that low frequency US (20 kHz) can induce a probe release much more efficiently than using high frequency waves (3 MHz), and, very important, through the application of US pulses of specific length and intensity the probe release is strongly dependent on the mechanical and chemical properties of the nanocarrier membrane. The in vitro work was followed by an ex vivo and in vivo (on a xenografted B16 melanoma mouse model) MRI study that successfully demonstrated the potential of this approach to visualize the probe release following a local US application. The possibility to selectively trigger the release of the imaging reporter, as well as a drug, from a mixture of different nanocarriers could open new and intriguing therapeutic schemes able to improve the overall efficacy of the pharmacological treatment.

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Self-assembled dextrin nanogels for biomedical applications.

Polymeric nanogels - also referred to as hydrogel nanoparticles, macromolecular micelles or polymeric nanoparticles - are emerging as promising drug carriers for therapeutic applications. These nanostructures hold versatility and properties suitable for the delivery of bioactive molecules, namely biopharmaceuticals. The polymer and the production methodology used are fundamental options. A particular challenge in this field is the development of preparation procedures avoiding the use of organic solvents or surfactants.

Dextrin is a very promising biomaterial, available in medical grade and accepted by the United States Food and Drug Administration for application in humans. In this work, dextrin was modified with long alkyl chains to produce an amphiphilic molecule. Dextrin-VA-SC16 (dexC16) has a hydrophilic dextrin backbone with grafted acrylate groups (VA), substituted with hydrophobic 1-hexadecanethiol (SC16). A versatile synthetic method was developed allowing control of the dextrin degree of substitution with the hydrophobic chains. Upon dispersion in water, dexC16 self-assembles through association of the hydrophobic alkyl chains, originating nanoparticles. The hydrophobic chains, randomly distributed along the polymer backbone, promote the formation of hydrophobic domains within the nanoparticles. Colloidally stable nanoparticles, with an average diameter of about 20-50 nm and a critical micelle concentration around 0.01 mg/mL, were obtained. The more substituted polymer forms more densely packed hydrophobic domains, such that the colloidal stability (in water or phosphate buffered saline solution) of nanoparticles is increased. The nanoparticles have a slightly higher size when prepared in buffer (irrespective of the pH) or in the presence of a salt or urea (irrespective of the concentration).

The uptake of nanoparticles by cells of the mononuclear phagocytic system limits its use as colloidal drug carriers, reducing the blood circulation time and the ability to reach biological targets. The interaction between dextrin nanoparticles and murine bone marrow-derived macrophages was evaluated in vitro by confocal laser scanning microscopy and fluorescence activated cell sorting. Fluorescein-labelled nanoparticles were used to assess the phagocytic uptake and blood clearance after intravenous injection. Cytotoxicity and nitric oxide production were studied, using the MTT assay and the Griess method, respectively. The results show that the nanoparticles are not cytotoxic and do not stimulate the production of nitric oxide by macrophages, in the range of concentrations studied. Nanoparticles are phagocytosed by macrophages and are detected inside the cells, concentrated in cellular organelles. The blood clearance study showed that the blood removal of the nanoparticles occurs with a more pronounced rate in the first 3 h after administration, about 30% of the material remaining in systemic circulation at this stage. The tissue distribution, after intravenous injection in Wistar rats, was evaluated using functionalized nanoparticles with a DOTA metal chelator and subsequently labelled with the gama-emitting 153Sm3+ radioisotope. The blood clearance rate and organ biodistribution of radioactively labelled nanoparticles was analysed, using materials both with and without poly(ethylene glycol) surface coating. The dexC16 nanoparticles display a characteristic biodistribution profile, being mainly taken up by the organs of the mononuclear phagocyte system - liver and spleen. The blood circulation time extends to several hours – as observed using the fluorescein-labelled nanoparticles, although the concentration is halved in about 1 hour. The functionalization of the nanoparticles with PEG 5,000 in this formulation improves their circulation time in the bloodstream and reduces the accumulation in the liver and spleen.

In order to explore the ability of dextrin nanoparticles as a drug carrier, curcumin was used as a model hydrophobic drug. Curcumin is a natural polyphenol with anti-oxidative, anti-inflammatory and anti-cancer properties. However, its therapeutical potential is substantially hindered by the rather low water solubility and bioavailability. Dextrin nanoparticles were tested as a curcumin carrier, allowing its dispersion in water, improving stability, and controlling release profile.

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Delivery of siRNA using iron oxide conjugated plasmid DNA nanoparticles for targeting brain tumors.
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Nanoparticles coated with single stranded DNA have been shown to efficiently hybridize to targets of complementary DNA and have recently been introduced as carriers for DNA/RNA delivery. We have previously shown that systemic injection of plasmid expressing siRNA against MMP-2 mRNA decreased tumor growth in vivo in an intracranial tumor model. However, non-targeted delivery also leads to even distribution throughout the body and can cause negative side effects and reduces the efficacy of therapeutics. In the present study we used super paramagnetic iron oxide nanoparticles conjugated with plasmid expressing Luc tagged MMP-2 siRNA followed by chitosan coating to target them to the tumor site. Plasmid expressing MMP-2 siRNA tagged to luc-coated iron oxide nanoparticles (MMP-2LFeNPs) positively identifies MMP-2 mRNA in cultured glioblastoma cells as determined by a decrease in MMP-2 mRNA and protein levels compared to cells treated with iron particles coated with plasmid expressing luciferase gene alone (LFeNPs). These particles were injected into mice with prestablished subcutaneous and intracranial tumor using U87 and SNB19 glioblastoma cell respectively, through the tail vein. The particles were directed towards the tumor site by means of external magnets. Successful tumor homing in vivo was observed by luciferase imaging. MMP-2LFeNPs accumulation in tissue sections was evaluated with in vivo imaging, green fluorescent protein as a tumor marker, and luciferase expression to determine nanoparticle localization at the tumor site. The study proved the efficacy of chitosan-coated iron oxide nanoparticles as an efficient delivery system for plasmid expressing siRNA against intracranial glioblastoma tumors.

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IDENTIFICATION OF BIOMARKERS IN COLORECTAL CANCER (PRE- & POST-CHEMOTHERAPY) BY NUCLEIC ACIDS PROGRAMMABLE PROTEIN MICROARRAYS (NAPPA), IFISH AND SNPs APPROACHES

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Biomarkers, particularly those with strong positive and negative predictive value, have many potential uses in the diagnosis and treatment of cancer, including monitoring treatment success, indicating disease progression and detecting early disease. One potentially powerful approach to finding biomarkers is to exploit patients' own immune systems, which produce humoral responses to cancer antigens released by their tumors due to alterations in protein expression, mutation, degradation, or localization. Antibodies to tumor antigens have been detected as early as several years before the clinical appearance of cancer. Although the specificity for these responses is high, typically only 5-20% of patients demonstrate a response to any given antigen, which has limited the usefulness of single antigen responses as biomarkers. The recent development of protein microarrays may offer an ideal tool for screening for immune response to tumor antigens. These arrays offer the advantage that hundreds to thousands of different proteins can be printed and screened simultaneously and only require a few microliters of serum per assay.
Prof. LaBaer´s group (Harvard Institute of Proteomics) has developed a novel method for producing protein microarrays called nucleic acid programmable protein arrays (NAPPA) that avoids the need to express and purify the proteins by substituting the printing of cDNAs on the arrays, which are then transcribed and translated in situ as needed at the time of the assay. NAPPA has been used successfully to map the pairwise protein interactions of the human DNA replication complex. Here, we propose adapting the NAPPA protein microarray technology for use in the rapid and efficient screening of sera from cancer patients for antibodies to 1000 known and potential tumor antigens in a multiplex format in order to better characterize the immune response to known tumor antigens, identify new informative tumor antigens and evaluate the value of using patterns of tumor antigen immune responses as biomarkers in Colorectal Cancer. For the validation of the possible biomarkers found in 20 different patients (pre- & post-chemotherapy), currently we are using iFISH and SNPs approaches with the main goal to correlate genomics and functional proteomics.

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A new Gd(III)-complex based system for the MR molecular imaging of MMPs in Multiple Sclerosis lesions.
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Matrix Metalloproteinases (MMPs) constitute a family of endoproteases that exert their function in the extracellular matrix and are differentially expressed in tissues or pathological states, such as tumors and neurodegenerative disease (RR-MS). The assessment of the activity of a selected panel of MMPs in a given tissue or anatomical district would therefore be of great value for the typization and staging of the pathological process. If such an assessment could be done in vivo by imaging techniques, its diagnostic content would be even greater. MRI is one of the most clinically relevant imaging techniques, because of its great spatial resolution (<100 um with modern high field equipment), lack of invasiveness (no ionizing radiation) and possibility to image soft tissues at any depth within the body. Contrast agents (CAs) based on paramagnetic materials are usually employed to enhance image contrast.
Recently, a way to the MR Molecular Imaging of MMPs has been presented based upon a MMPs-cleavable peptide functionalized at the N-terminus with a Gd(III)-DOTA chelate as the reporter unit, in which the MMP activity can be detected on the basis of the differences in wash-out kinetics between the Gd(III) probe cleaved by MMPs and the intact probe.
The CAs developed in this work are based upon a MMP-cleavable peptide functionalized with a Gd(III)-DOTA as the reporter unit, and with a hydrophobic alkyl chain for binding to poly-Î²-CD. Compound K11 contains the PLGLWAR peptide sequence ending with a C11 alkyl chain. K11 appeared to be poorly recognized by MMPs likely because of the steric hindrance of the DOTA macrocycle directly bound to the Proline residue, known to be essential for MMP recognition.
To make K11 a better substrate for MMP-1/MMP-12 the DOTA moiety has been moved far away from the proline by inserting a GVV tripeptide strip. Furthermore a polyethylenglycol-based spacer between the N-terminal of the peptide and the DOTA unit has been added to increase the hydrophilicity of the molecule (compound K11N). Both K11 and K11N interact with poly-Î²-CD with association constants (Ka) of 300 M and 500 M, respectively. K11N can be cleaved by MMP-12/ MMP-1 either in the free form or as the inclusion complex with poly-Î²-CD.

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Integrin-Targeted, Smart Nanocomplexes for Tumour Specific Delivery and Therapy by Systemic Administration

We are developing Receptor-Targeted Nanocomplex (RTN) formulations for systemic delivery to target metastatic tumours, comprising peptides and lipids with environmentally sensitive chemical linkers, which are cleavable within the intracellular environment but stable extracellularly. These properties, it was hypothesised, would promote particle disassembly within the cells and higher transfection efficiencies. RTNs consist of a PEGylated lipid (ME42/DOPE), an RGD-targeting peptide (ME27) and plasmid DNA. PEGylation of the lipid stabilizes the nanocomplex particles while the ester linkage allows esterase cleavage of the PEG moiety within the endosome. The peptide (ME27) comprises an integrin-targeting cyclic RGD domain and a sixteen lysine domain for DNA binding, separated by a four-amino acid linker sequence which is cleavable by the endosomal enzymes furin and cathepsin B. Endosomal cleavage of the peptide targeting ligand, should promote disengagement from the integrin receptor at the endosomal stage, and thus enable trafficking to the cytoplasm.

The objective of this study was to evaluate delivery of the RTN (ME42/DOPE:ME27) formulation to assess tumour efficiency and specificity of delivery, integrin-targeted transfection, and biodistribution, and therapeutic efficacy. The cleavable RTN formulation (ME42/DOPE:ME27) demonstrated highest transfection efficiencies after systemic delivery to neuroblastoma tumours with a transfection efficiency of 60% of tumour cells. Plasmid DNA and luciferase reporter gene expression delivered by the RTN were both predominantly located to the tumour, with expression highly restricted to the tumour. RTN transfection efficiency was shown to be enhanced two fold by integrin-targeting peptides. Therapeutic efficacy was evaluated in neuroblastoma tumours using therapeutic RTNs containing the interleukin-2 (IL-2) and IL-12 genes, which is a highly immunostimulatory combination of genes particularly effective in promoting differentiation and proliferation of Th1 cytotoxic T cells. Tumour growth was reduced approximately 75% after intravenous administration of therapeutic RTNs compared with controls in the first 11 days post-engraftment ($p<0.05$). Extensive leukocyte infiltration, decreased vascularisation and increased necrotic areas were observed in the tumours. One third of mice treated with IL2/IL12 RTNs survived long-term (90 days), with complete tumour eradication. Splenocytes from rechallenged mice displayed enhanced IL-2 production following Neuro-2A co-culture, which, together with infiltration studies, suggested a cytotoxic-T cell mediated tumour-rejection process. We conclude that “smart” RTNs target tumours avoid clearance by first-pass organs, to specifically and efficiently deliver genes to tumours by systemic administration. Delivery of IL2/IL12 cytokine genes was sufficient to retard and eradicate established neuroblastoma tumours in mice.

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South Africa currently has the highest incidence of TB per 100 000 people in the world. In 2007 alone 112 000 people died of TB in South Africa, of which 94 000 were co-infected with HIV. Although TB treatments exist, poor patient treatment compliance and drug resistance pose a great challenge to TB treatment programs worldwide. To improve the current inadequate therapeutic management of TB, a polymeric anti-TB nanodrug delivery system, for anti-TB drugs, was developed that could enable entry, targeting, sustained release for longer periods and uptake of the antibiotics in the cells, hence reducing the dose frequency and simultaneously improve patient compliance.

The aim was to prepare functionalised polymeric nano drug delivery vehicles to target TB infected macrophage cells. Successful nano encapsulation of anti-TB drugs and a targeting agent, mycolic acid (MA) was achieved. MA (a lipid molecule on the cell wall of M.tb,) was explored due to its cholesteroid properties that could attract it to the infected macrophages/foam cells. The nanoparticles were characterized and subjected to in vitro analyses in order to determine their uptake, localization and cytotoxicity in different cell lines. In another approach targeting was achieved via attaching nucleic acid aptamers specific for the mannose receptor (MR), which is significantly over-expressed during the activation of the macrophages in the presence of M tb onto the surface of drug-carrying PLGA nanoparticles. Uptake of the MA PLGA nanopaprticles was achieved where little co localization was observed with endocytic markers, indicating that they could be localized on the cytosol. Vehicles of the particles were also observed in the cell membrane of these cells. The uptake of the aptamers to THP-1 cells also illustrated the feasibility of using the nucleic acid species for active targeted drug delivery. The success of these two approaches of anti-TB drug targeting will greatly addresse the challenges of poor bioavailability, reduced efficacy and adverse side effects for diseases such as TB.

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Biodistribution of RGD-targeted liposomes containing doxorubicin in mice bearing C-26 colon Carcinoma

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Drug therapy for the treatment of tumors is often limited by a narrow therapeutic index. One approach that overcomes this limitation is the active targeting of tumors with particulate drug carriers containing chemotherapeutic drugs among which nanoliposomes are of the most promising delivery vehicles. The derivatization of nano-sized drug loaded liposomes with a ligand leads to the selective targeting of the particulate to selected areas in body, thereby focusing drug delivery. RGD peptides targeting integrins are promising ligands for the generation of vascular targeting agents. The aim of this study was to evaluate the biodistribution behavior of stealth RGD-targeted liposomes containing doxorubicin (Dox) in mice bearing c26 colon carcinoma. Nanoliposomes consisted of HSPC (hydrogenated phosphatidylcholine), cholesterol, mPEG2000-DSPE (methoxy-polyethylene glycol 2000-distearoyl phosphatidyl ethanolamine) were prepared by thin film method plus extrusion technique and Dox were remotely loaded using ammonium sulfate gradient. Then the RGD peptides were attached to the distal end of maleimide functionalized mPEG2000-DSPE. RGD peptides were consisted of 3 cyclic RGD based peptides (RGD1-Lip-Dox, RGD2-Lip-Dox, RGD3-Lip-Dox) and a cyclic RAD peptide (RAD-Lip-Dox) as negative control. All the preparations were characterized for their size, zeta potential, percent Dox encapsulation and percent RGD-peptides linking. Then the preparations and CaelyxTM (a commercial liposome Dox formulation) were administered intravenously to mice bearing c26 colon carcinoma and the fluorescence of Dox were assayed in different organs (heart, liver, kidney, lung, spleen, muscle and tumor) as well as in serum at different time points after administration. Average diameters of RGD-Lip-Dox preparations were 100 nm and percent RGD-peptides linking were near to 100%. Biodistribution studies indicated that although RGD-Lip-Dox preparations clear from the blood faster compared to CaelyxTM, no significant differences were observed in tumor accumulation of RGD1-Lip-Dox, RGD2-Lip-Dox and CaelyxTM. However, RGD3-Lip-Dox was accumulated in tumor significantly more than CaelyxTM. The interaction of RGD-Lip-Dox preparations with HUVEC cells, which overexpress integrins, were also evaluated in different incubation times. The association pattern of RGD3-Lip-Dox with cells indicated faster and higher interaction of this preparation with HUVEC cells. The results of this study indicate that RGD3 is a promising ligand for active targeting of liposomal-Dox to solid tumor. Evaluations of anti-tumor efficacy of the RGD-Lip-Dox preparations in mice bearing c26 colon carcinoma are in process.

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NANOTECHNOLOGY APPROACHES FOR HIGH-THROUGHPUT DETERMINATION OF SMALL KINASE INHIBITORS ACTIVITY ON CKIT.

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Human KIT is a proto-oncogene that encodes for a trans-membrane receptor (cKit) with intrinsic tyrosine-kinase (TK) activity which functions as the receptor for Stem Cell Factor (SCF). Expression of the cKit protein has been reported in a wide variety of cells, including mast cells, hematopoietic progenitors, germ cells or melanocytes. Alterations on Kit expression/activity are associated with several hematopoietic disorders, grastrointestinal stromal tumours (GIST), piebaldism, among other diseases.

At present, multiple cKIT mutations have been reported, many of them associated with constitutional Kit phosphorylation and downstream activation, independent of the SCF binding. The recent advances in the field of molecular-targeted therapy allow us to select drugs on the basis of specific molecular abnormalities. Nevertheless, at present this studies are costly, have long response times and, in many diseases are difficult to since there's a limited availability of samples. Here, we propose a high-throughput method for screening different drugs that it will be used in Kit-related disorders, whether it's mutated or not.

We designed and prepared a Protein Microarray (NAPPA), based in the technology developed at Josh LaBaer Laboratory (Harvard Institute of Proteomic, Boston, EEUU), where we have spotted wild type cKit and all the cKit mutations described in the literature. Using kinase NAPPA arrays we showed that the kinases on the array display autophosphorylation activity. To demonstrate this, we used anti-phosphotyrosines (pTyr) antibodies to detect pTyr on the kinases on the arrays; these pTyr disappeared after treatment with phosphatase and then returned after incubation in kinase buffer and ATP.

To determine if the kinase activity could be affected by inhibitors, the autophosphorylation activity of the protein was also evaluated when the reactions were performed in the presence of global kinase inhibitors such as ADP and Staurosporine. In this case, the observed reduction of the kinase activity suggested that the proteins on the array can be inhibited by general kinase inhibitors, as expected. Ongoing experiments are using kinase NAPPA arrays to study the effect of specific kinase inhibitors (small molecules) on the kinase activity, and determine the small molecule selectivity and efficiency among the different mutated on not cKit kinases, all performed in a single experiment. This method may provide a high throughput rapid approach for evaluating drug selectivity without having the need for human patient samples.

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Epithelial sodium channels determine the nanomechanics of endothelial cells

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Nanomechanical properties of endothelial cells are critical parameters in vascular dysfunction. Foremost, the stiffness of endothelial cells plays a crucial role in the regulation of vascular function as it controls the production of the endothelium-derived vasodilator nitric oxide (NO). Increase in endothelial cell stiffness and thus reduced NO release results in the contraction of vascular smooth muscle cells, a rise in peripheral resistance and high blood pressure. The nanomechanical properties of the endothelium are therefore pivotal for the pathogenesis of hypertension. One pathogenetic factor is the mineralocorticoid hormone aldosterone as it leads to an increase in endothelial stiffness. Moreover, the hormone has been shown to regulate the abundance of the endothelial sodium channel (ENaC) in the plasma membrane of endothelial cells. Since aldosterone-induced cell stiffening can be prevented by the ENaC-specific functional blocker amiloride, it is postulated that ENaC controls cell stiffness.
To test this, the influence of alterations in ENaC cell surface density on the mechanical stiffness of endothelial cells was determined. To this end we used the tip of an atomic force microscope (AFM) as a mechanical sensor to measure the deformability of a stably transfected αENaC siRNA endothelial cells. In these cells, αENaC, the subunit indispensable for proper ENaC function, is knocked down. Nanoindentation measurements demonstrate a decrease in mechanical stiffness by about 35 % compared to mock-transfected control cells. In another series of experiments we studied the effect of overexpressing fluorescent ENaC-eGFP in living endothelial cells using a hybrid AFM-fluorescence-microscope. ENaC upregulation increases cell stiffness by about 45 % compared to non-transfected cells. Our findings are supported by Western blotting and single ENaC molecule detection, using appropriate antibodies against αENaC.
We conclude that in endothelial cells ENaC molecules in the plasma membrane control mechanical stiffness and thus participate in the control of blood pressure.

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TNF-alpha knockdown model system for polymeric siRNA delivery using an LPS-induced macrophage cell line.

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Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disorder characterized by joint inflammation, immune cell infiltration of the synovia, and cartilage/bone destruction. Tumor necrosis factor alpha (TNF-alpha) is thought to be one of the major players in this complex interplay between cellular cytokines, which maintain the inflamed state of the joint. Therefore silencing TNF-alpha expression by induction of RNAi seems to be a promising strategy for future RA treatment.

As macrophages are thought to play an important role in RA the murine macrophage cell line RAW264.7 was chosen as an in vitro model to test and compare the delivery efficiencies of the different polymeric siRNA delivery systems. TNF-alpha expression was induced with Lipopolysaccharide (LPS), and the optimal LPS concentration was found to be 50 ng/mL. The TNF-alpha expression levels were determined on the protein level by flowcytometric detection of intracellular TNF-alpha expression. Therefore the assay was optimized with respect to i) the applied protein secretion blocking reagent ii) blocking procedure and iii) antibody concentration. Additionally the TNF-alpha mRNA levels were quantified by qRT-PCR and normalized to several housekeeping genes (beta-actin, Gusb (glucuronidase-beta) and Hprt (hypoxanthine-guanine phosphoribosyltransferase).

Future studies will compare the gene silencing activity of siRNA-loaded polymers including PLGA (poly(D,L-lactide-co-glycolide)), TMC (Trimethylated chitosan), nanogels and two polymers with a pHPMA backbone (poly(N-2-hydroxypropyl methacrylamide), namely pHPMA-DMAE (2-dimethylamino-ethyl ester) and pHPMA-MPPM (1-methyl-2-piperidine methanol). In addition data regarding polymer-induced non-specific side effects (mainly cytokine expression patterns) will be addressed using the above mentioned techniques.

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Polymer Based Biosensor for Rapid Electrochemical Detection of Human Cytomegalovirus Infection in Human Fibroblast Cells

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The demand in the field of medical diagnostics for simple, cost efficient and disposable devices is growing. Here, we present a label-free, all-polymer electrochemical biosensor for diagnosis of an acute viral disease.

The dynamics of viral infection in human cell culture were investigated in an all-polymer micro fluidic system on conductive polymer microelectrodes by electrochemical impedance spectroscopy (EIS) and video time lapse microscopy.

EIS is a sensitive, real time electrochemical technique to measure the interaction between a cell monolayer and the electrode surface (Giaever et al., 1986). The measured impedance reflects changes in the attachment and the morphology of cells, and reaches its maximum when the electrode surface is completely covered. The technique is very sensitive to small changes in the cell membrane capacitance, and given that cell morphology is an indicator of the well-being of cells, it is a suitable non-invasive technique for rapid electrochemical detection of virus infection in human cells. Viral infections typically induce degenerative morphological changes in cell cultures to produce a decline in resistance and an increase in capacitance in the EIS readout (McCoy et al., 2005; Campbell et al., 2007).

Using EIS, we have demonstrated that infection with human cytomegalovirus (HCMV) of human foreskin fibroblast cells is detectable within few hours post infection – several hours before the cytopathic effect is apparent. The immediate cell response to virus is mirrored by drastic fluctuations in the impedance. The impedance peaks within one hour post infection to reach a local maximum and then gradually decreases to a local minimum in the hour that follows, presumably reflecting the inter- and intra-remodeling events caused by the virus. These initial cellular changes are not observable in a microscope. Video time lapse microscopy studies of cell cultures show that the cytopathic effect of the virus manifests itself circa 10 hours post infection depending on the multiplicity of infection.

The HCMV infection study in human cells indicates that EIS is a very powerful tool for rapid, real time quantification of virus infection in cell culture at biologically relevant concentrations. Employing EIS, the time frame of the study can be reduced five times compared to conventional inspection methods, such as visual evaluation of cell morphology by microscopy.

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Delivery of siRNA using iron oxide conjugated plasmid DNA nanoparticles for targeting brain tumors.
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Nanoparticles coated with single stranded DNA have been shown to efficiently hybridize to targets of complementary DNA and have recently been introduced as carriers for DNA/RNA delivery. We have previously shown that systemic injection of plasmid expressing siRNA against MMP-2 mRNA decreased tumor growth in vivo in an intracranial tumor model. However, non-targeted delivery can also lead to even distribution throughout the body and reduces the efficacy of therapeutics. In the present study we used super paramagnetic iron oxide nanoparticles conjugated with plasmid expressing Luc tagged MMP-2 siRNA followed by chitosan coating to target them to the tumor site. Plasmid expressing MMP-2 siRNA tagged to luc-coated iron oxide nanoparticles (MMP-2LFeNPs) positively identifies MMP-2 mRNA in cultured glioblastoma cells as determined by a decrease in MMP-2 mRNA and protein levels compared to cells treated with iron particles coated with plasmid expressing luciferase gene alone (LFeNPs). These particles were injected into mice with prestablished subcutaneous and intracranial tumor using U87 and SNB19 glioblastoma cell respectively, through the tail vein. The particles were directed towards the tumor site by means of external magnets. Successful tumor homing in vivo was observed by luciferase imaging. MMP-2LFeNPs accumulation in tissue sections was evaluated with in vivo imaging, green fluorescent protein as a tumor marker, and luciferase expression to determine nanoparticle localization at the tumor site. The study proved the efficacy of chitosan-coated iron oxide nanoparticles as an efficient delivery system for plasmid expressing siRNA against intracranial glioblastoma tumors.

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Image-guided and tumor-targeted nanomedicines for radiochemotherapy

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Introduction: Drug targeting systems are nanometer-sized carrier materials designed for improving the biodistribution of systemically applied (chemo-) therapeutics. Reasoning that I) the temporal and spatial interaction between i.v. applied chemotherapy and clinically relevant fractionated radiotherapy is suboptimal, and that II) drug targeting systems are able to improve the temporal and spatial parameters of this interaction, we have set out to evaluate the potential of 'carrier-based radiochemotherapy'.

Methods: HPMA copolymers were used as a model drug targeting system, doxorubicin and gemcitabine as model drugs, and the syngeneic and radio- and chemoresistant Dunning AT1 rat prostate carcinoma as a model tumor model. Magnetic resonance imaging, γ-scintigraphy, fluorescence microscopy and HPLC were used to evaluate the biodistribution and the tumor accumulation of the copolymers, and clinically relevant regimens of radiotherapy and chemotherapy to evaluate their efficacy-enhancing effects.

Results: First, the polymeric drug carriers were shown to circulate for prolonged periods of time, to localize to tumors both effectively and selectively, and to improve the tumor-directed delivery of low molecular weight agents. Subsequently, they were then shown to interact synergistically with radiotherapy, with radiotherapy increasing the tumor accumulation of the copolymers, and with the copolymers increasing the therapeutic index of radiochemotherapy (both for doxorubicin and for gemcitabine).

Conclusion: Based on these findings, and on the fact that its principles are likely broadly applicable, we propose carrier-based radiochemotherapy as a novel, a rational, a translational and a relatively straightforward concept for treating advanced solid malignancies.

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Use of fluorescent gold nanocrystals-labelled proteins in cell experiments

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Fluorescent labelling techniques have been used extensively in both biological research (in vivo imaging & targeting) and clinical diagnosis because of its high sensitivity, simplicity, and diversity. However, they present a number of issues when applied to biological systems, including large physical size, strong fluorescence intermittency on all time scales, and cell toxicity. Additionally, due to penetration and background issues, the development of bright emitters not only in the visible but also in the near-infrared is needed to eventually enable intracellular single molecule studies with minimal perturbation.
A new family of fluorescent biomarkers, noble metal (Gold, Silver) nanoclusters (NCs), have drawn a considerable research interest in the past 5 years which put them in a complete challenge with the best biolabelling existing, such as semi-conductor quantum dots, organic dye-doped nanoparticles or metallic particles. These NCs have been prepared in different templates such as polymeres, polyelectrolytes or proteins with sizes below 2 nm and have fluorescent emission based on the particular size-dependent properties1. We incorporate fluorescent NCs emitting in the red region inside an inorganic matrix at different concentration and demonstrate the role of silica as a protective host against solvent effect and photobleaching. Cell experiments confirm also the potential of this nanomaterial for imaging application.

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Size and Ligand Dependent Cytotoxicity of Gold Nanoparticles

Gold nanoparticles (AuNPs) exhibit size dependent distinct physical, chemical and biological properties from their bulk counterparts and are therefore of great interest for use in biomedicine as imaging tools, phototherapy agents and gene delivery systems. These promising applications are confronted by relatively few studies concerning possible adverse effects on living organisms to date.

In a first study, we investigated the cytotoxicity of AuNPs with different sizes in a range between 0.8 nm to 15 nm, all stabilised by a water soluble triphenylphosphine ligand (TPPMS). The nanoparticles were characterised by means of UV/Vis, SEM or TEM, elemental analysis and AAS. The cytotoxicity was tested by MTT assay in four different cell lines. In all these, AuNPs with a mean diameter of 1.4 nm proved to be most toxic. In contrast, AuNPs with a size of 15 nm were found to be non-toxic at up to a 60-fold higher concentration. In a propidium iodide/annexin V double staining and subsequent flow cytometry experiment, 1.4 nm particles caused predominantly necrosis within 48 h while 1.2 nm particles provoked predominantly apoptosis.

A further investigation showed that the cytotoxicity of 1.4 nm sized AuNPs is related to oxidative stress. The addition of a variety of antioxidants such as glutathione (GSH) and N-Acetylcysteine (NAC) could significantly reduce the toxicity. GSH stabilised AuNPs were clearly less cytotoxic than phosphine stabilised ones.

A DNA gene chip analysis showed up-regulation of stress-related genes. Furthermore, it was found that size and ligand chemistry of the AuNPs play a crucial role, as 15 nm sized particles and GSH stabilised, 1.1 nm sized particles didn't induce the same cell reactions.

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In Vitro Model of Inflammatory Bowel Disease as Screening Tool for Anti-inflammatory Drug Formulations

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Classical therapy of inflammatory bowel disease (IBD) with steroids or immunomodulators is often inefficient mainly due to limited drug release time and enhanced elimination of drug carriers as result of diarrhoea. Nanoparticles have been shown to be promising formulation strategies to overcome this problem[1]. However, in the past in vitro testing of novel drugs and formulations for IBD was usually conducted in healthy epithelial cell culture models (e.g. Caco-2) as there were no suitable in vitro models of the inflamed intestinal barrier. We herein report on the establishment and characterization of such an advanced in vitro model and its successful application for in vitro screening of novel budesonide formulations for IBD therapy.

The model consists of Caco-2 cells representing the intestinal epithelium and blood derived macrophages and dendritic cells as the immunocompetent cells of the intestinal barrier[2]. Inflammation is triggered by addition of pro-inflammatory cytokine interleukin-1β[3], which induces increased release of pro-inflammatory marker interleukin-8, re-arrangement of tight junctional proteins accompanied by reduction of barrier function and increased mucus production. Various budesonide formulations (free budesonide solution, budesonide-loaded PLGA nanoparticles, or budesonide-loaded liposomes) were applied to the inflamed model and IL-8 release was measured for inflammation monitoring. Transepithelial electrical resistance (TEER) was monitored to observe recovery in epithelial barrier function. Furthermore particle deposition was observed using confocal laser scanning microscopy.
Both budesonide in solution and encapsulated in PLGA nanoparticles were able to restore the epithelial barrier function and decreased IL-8 protein release within 24 hours after treatment, indicating reduced inflammation. PLGA-budesonide treated cells maintained the reduction of IL-8 protein after 48h while free budesonide treated cells showed a rebound. Liposomal budesonide negatively affected the co-culture further reducing TEER value and increasing IL-8 release rate compared to the control. PLGA nanoparticles were observed to accumulate intercellularly between epithelial cells while liposomes accumulated in the basolateral side of the model, co-localized with macrophages and dendritic cells.

In conclusions, the PLGA formulation seems to be more efficient and suitable for inflamed tissue treatment compared to liposomes and should be further evaluated in a colitis animal model. The in vitro model of the inflamed intestinal mucosa has the convenience of an in vitro study while at the same time reflecting the complex pathophysiological changes in inflamed mucosal tissue and thus can be used to screen anti-inflammatory drugs and formulations before in vivo testing.

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Novel method of controlled and inducible AFM tip functionalisation with biomolecules.

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The infection with Herpes Simplex Virus type 1 (HSV-1) proceeds via a stage when the viral capsid docks at the nuclear pore complex (NPC) and translocates its genome into the nucleus of a non-dividing neuronal cell. It is generally believed that for this to occur the capsid needs to assume a particular orientation at the pore when the so called “portal protein” UL6 faces the NPC central channel. Nevertheless, experimental evidence in support of this hypothesis is still lacking.We plan to test this hypothesis by picking individual capsids from the surface in a portal down orientation and probing their interactions with the nuclear envelope. This, however, requires a novel strategy of inducible functionalisation of the Atomic Force Microscopy (AFM) tips with biomolecules. Development and optimisation of such an experimental approach was performed on surface immobilised bovine serum albumin and polystyrene beads of the size comparable to that of the capsids. Optimal control of the process was achieved by employing photoreactive crosslinker covalently bound to the surface of the AFM tip. Activation of the crosslinker could then be performed on demand during the process of imaging by illuminating the tip with UV light. Success of the immobilisation process could then be directly monitored by the change in sample topography or tip-sample interactions. Our results show that the tips have been functionalised with the crosslinker as indicated by marked increase in the tip hydrophobicity. Subsequent photoactivation of the linker has enabled us to detach single protein molecules from the hydrophobic surface. The method has also been successfully applied to aminofunctionalised polystyrene beads. We believe that the developed method is capable of controlled, inducible and potentially orientation-specific immobilisation of HSV-1 capsids on the AFM tips for investigation of their interactions with the NPCs.

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A commercial and technological comparison of industrial nano-sized drug delivery systems
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Advanced drug delivery aims to alter the therapeutic index of drugs via association with drug delivery systems (DDS). Nano-sized DDS (NDDS) are increasingly evaluated within academia and industry. An in-depth market search revealed that at present 126 companies are involved in the development of more than 200 NDDS. Twenty-two NDDS are currently on the market, whereas the greater part of NDDS is still in preclinical development. The majority of these NDDS have cancer as target indication, due to high medical need and requirement for better therapies.

The technological features of a selection of NDDS were evaluated by comparing the formulation features, pharmacokinetics, pharmacodynamics and clinical results as described in scientific publications. Eight NDDS were compared to free drug in clinical trials with regard to pharmacokinetic and therapeutic outcome. Taxoprexin and Myocet were NDDS that changed the pharmacokinetics of the encapsulated drugs positively, while they did not improve overall survival of treated patients. Doxil positively shifted the biodistribution of doxorubicin and generated an improved clinical benefit. Abraxane and Genexol demonstrated, despite an equal pharmacokinetic profile as compared to the free drug paclitaxel, an improved patient survival albeit evidently not in all situations.

These results indicate clearly that the pharmacokinetic profiles of entrapped drugs can not be used to accurately predict the clinical outcome of NDDS. Entrapment in NDDS did however shift the adverse effect profile to some extent. NDDS that successfully obtained marketing authorization appear to be relatively simple formulations, making them easier to develop and to characterize.

A better overview of developments and results, including failed projects, is needed to obtain detailed and realistic insights in the clinical value of NDDS. In addition, more collaboration in the scientific community has to generate consensus on the characteristics of an ideal NDDS, based on indication and NDDS type. This knowledge is anticipated to speed up the industrial development of NDDS.

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Solid Lipid Nanoparticles Containing Oryzalin for the Treatment of Leishmaniasis

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Dinitroanilines, such as trifluralin and oryzalin (ORZ), are tubulin-binding agents that display antimitotic activity on Leishmania sp. and lack of toxicity to animals or humans. These facts make dinitroanilines potential efficient drugs, but its therapeutic use is limited by low aqueous solubility and low vapour pressure. So far, due to its hydrophobicity ORZ could not be formulated, at therapeutic doses, in a vehicle suitable for i. v. administration. This key drawback may be overcome by the use of solid lipid nanoparticles (SLN). The aim of this work is the development of methods and optimization of SLN formulations for the incorporation of ORZ with high encapsulation parameters and stability.

For SLN preparation an emulsification/solvent evaporation method was optimized. Briefly, three SLN formulations were developed containing tripalmitin (5% w/v) as the core lipid and mixtures of several surfactants (egg lecithin 2,4%, sodium deoxycholate 0.6% and Tween20 0.5% w/v) for particle stabilization. All SLN prepared by this method presented sizes in the nano range (100-200 nm) with highly homogenous populations (Polidistersity index <0,3) and negative surface charge (-33 to -39 mV).

At least 5% w/w ORZ was evaluated for incorporation in these SLN formulations. For all formulations the results showed an encapsulation efficiency of 80-90%. Drug incorporation resulted in a small increase in particles mean size diameter although no surface charge variation was observed.

All formulations (with and without ORZ) were stable in terms of mean size diameter and surface charge at 4 ÂºC during at least 2 months. In stability studies in a freeze dried form SLN with lecithin as a surfactant proved to be formulation with higher stability, even without cryoprotector (5% w/w sacarose). For this formulations no significant mean size diameter or surface size variation were observed and ORZ yield was approximately 85% after rehydration of the freeze dried formulation.

Formulation sterilization by autoclaving (121ÂºC/15 min) for all SLN formulations was also successfully tested. After the sterilization process, no significant size and surface charge variation as compared to non sterilized formulation were observed and ORZ yield was approximately 75%.

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Nanostructure Strontium- substituted Bioactive Glass Coatings for Medical Implants
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Introduction:
Metallic prosthetic implants are widely used to treat joint and skeletal injuries. However, some implants can fail because the metal alloys used do not bond with bone. Bioactive glass (BG) coatings may offer a solution to this problem as they form a strong bond with living tissue and have the added benefit that their dissolution ions stimulate cell activity. Strontium(Sr)can be substituted for calcium in BGs creating a material that may combine the bone remodelling benefits of Sr ions with the well-established bone stimulatory action of bioactive glass. The aim of this study is to develop Sr containing BG coatings on metallic surfaces to bond to an implant as an effective biomaterial choice for a range of bone regeneration therapies.
Materials and Methods:
BG in the system: SiO2-MgO-Na2O-K2O-ZnO-P2O5-CaO with 0, 10, and 50 of the Ca being replaced by Sr were prepared by a melt-quench route. A degradation study was carried out in simulated body fluids. Silicon [Si], Phosphorus [P], Calcium [Ca] and Sr were checked at different time periods. Produced glasses were combined with RPMI media. The human osteosarcoma cell line, Saos-2, was seeded in Sr substituted medium. Cell metabolic activity was measured using the tetrazole MTT. BGs were coated on to the surface of Ti6AL4V coupons and Saos-2 cells were seeded on BG coatings and viability was assessed with a Live/Dead stain.
Results:
Glass dissolution leads to a rise of all the ion concentrations in solution. MTT activity increased in all samples with time in culture until 14 days. Saos-2 cultured treated with Sr-substituted BGs had higher MTT activities than controls. Live/Dead staining showed that cells were alive on all coating materials.
Discussion and Conclusions:
Substituting strontium for calcium increases the dissolution of the glass releasing in more ions in solution. These ions may tend to enhance the metabolic activity of Saos-2 cells, which may lead to an increase in mineralization. As BGs are a well-studied biomaterial system capable of controlled ion release and are deliverable as coatings or scaffolds substitution of strontium for calcium in bioactive glasses may be an effective strategy for creating materials for bone repair/regeneration therapies.

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Formulation Studies on NSAID- containing SLN

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The non-steroidal anti-inflammatory drugs (NSAIDs) are widely used for the treatment of minor pain and for the management of oedema and tissue damage resulting from inflammatory joint disease. The major mechanism by which the NSAIDs elicit their therapeutic effects is inhibition of prostaglandin (PG) biosynthesis. Specifically NSAIDs competitively (for the most part) inhibit cyclooxygenases (COX-1 and COX-2), the enzymes that catalyze the synthesis of cyclic endoperoxides from arachidonic acid to form prostaglandins. Consequently, the use of NSAIDs can lead to different side effects, such as gastrointestinal (nausea, abdominal pain and gastric ulcer) and also hepatic problems.

Thus, it is important to develop topic use formulations regarding to NSAIDs use, as they have an active role in the inflamed area. The NSAIDs are insoluble in water and become clear the choice for encapsulation in Solid Lipid Nanoparticles (SLN).

SLN are the new generation of nanoparticulate active-substance vehicles and are attracting major attention as novel colloidal drug carriers for topical use. Compared with other vehicles such as creams, tinctures, and emulsions, SLN combine such advantages as controlled release, negligible skin irritation, and protection of active compounds. Moreover, their small particle size ensure that the nanoparticles are in close contact with the stratum corneum (SC), thus promoting the amount of the encapsulated agent which penetrates into the skin.

Lipid nanoparticles were prepared using the method of cold homogenization with solvent evaporation, using as a tensioactive polyvinil alcohol (PVA) and the lipid tripalmitin.

The optimization of the SLN was followed by changing the homogenization time, rate of homogenization, proportion of the tensioactive, the ratio of volume of lipid to tensioactive and drug's concentration. The size and zeta potential of the nanoparticles were used as parameters to evaluate the stability of SLN along all the process, as they have tendency to increase their size because of a possible aggregation.

In other to better dissolve the drug (NSAID) in the lipid (tripalmitin) an organic solvent (dichloromethane) has been used, and then mixing it drop by drop to the aqueous phase with the PVA), with a high-shear mixer (Silverson), followed by the evaporarion of the dichloromethane, in constant stearing, for 4hours.

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Multi-compartment vectors as novel drug delivery systems: selective activation of Tgamma-delta lymphocytes after zoledronic acid delivery

Multi-compartment nanoscopic carriers can be easily assembled by inducting the aggregation of anionic 'hybrid' niosomes by means of cationic biocompatible polyelectrolytes.

The resulting vesicle clusters, whose size and overall net charge can be easily controlled by varying the polyelectrolyte/particle charge ratio, show an interesting potential for multi-drug delivery. In this research a strong evidence for their effective use in vitro as multi-compartment vectors selectively directed toward monocyte/macrophage (MM) cells was provided. The study report on the interaction in vitro of hybrid niosomes, loaded with ZA, with different leucocyte subsets combining characteristics of niosomes and the versatility of their £`-PLL-induced aggregates.

The present study furnishes a first and clear evidence of a selective interaction with a subset of limphocytes, the monocyte/macrophages, of aggregates characterized by a well defined size and charge.

In this light, the multi-compartment £`-PLL niosome clusters, being potentially able to transport different substances within their different compartments, and with their intrinsic selectivity toward the macrophages, appear particularly suitable for implementing therapeutical strategies against the chronically infected macrophages. Particularly for HIV diseases, a strategy of intracellular drug delivery with a high degree of specificity, by reaching each single infected cell with the drug at therapeutic doses, could dramatically improve the efficacy of the therapy.

For this reasons, zoledronic acid (ZA), an immuno-modulating molecule, was chosen in this study as model of drug-cell interaction specific to leucocytes. It is known that V£^9V£_2 T-cells are key effector cells during infections diseases, being able to kill infected cells, to produce modulating and antiviral cytokines and to orchestrate the whole immune response. In this study the MM mediated activation of T£^£_ lymphocytes induced by Zoledronic acid (ZA) enhancement of a factor 103 when the ZA is intracellularly delivered through these carriers was showed.

Furthermore, the multi-compartment £`-PLL niosome clusters, with their intrinsic selectivity toward the macrophages, appear particularly suitable for implementing therapeutical strategies against the chronically infected macrophages.

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Atomic force microscopy reveals non-selective gating of the nuclear barrier

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The nuclear envelope is spanned at regular distances by nuclear pore complexes (NPCs). These multiprotein complexes selectively control the entire nucleoplasmic transport and limit the efficiency of gene therapy strongly. For the latter to proceed, a significant nuclear delivery rate of exogenously applied therapeutic nanoparticles (40-60nm) is necessary. The rate of nuclear delivery is marginal due to the exclusion of the nanoparticles from the NPC channel which is 10 nm wide but is able to dilate to 40 nm upon selective transport of cargo. Inducing NPC channel dilation could be crucial to increase nuclear delivery rate of nanoparticles. Here we show that cyclohexandiol, an amphiphatic alcohol and dexamethasone, a synthetic derivate of the physiologically and therapeutically critical glucocorticoids, lead directly to a reversible NPC dilation.

Structural details of the NPC dilation are analysed by means of atomic force microscopy and correlated with functional changes in nuclear envelope permeability. After exposure to cyclohexandiol NPCs were found dilated. NPC dilation was paralled by a nuclear envelope leakiness to a fluorescent macromolecule, 12 kDa FITC-Dextran, which is otherwise known to be excluded from NPC passage. 2 min after exposure to dexamethasone NPC were also found dilated. In addition, a yet unknown configuration, so-called giant pores, up to 300nm in diameter, was visualised. We conclude that dexamethasone and cyclohexandiol transiently open unspecific pathways for large macromolecules. Treatment with these reagents could potentially be useful for improving the efficiency of nuclear gene transfection.

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Multifunctional nanoplatform for in vivo and in vitro biomedical applications

Nanotechnology offers clear advantages over conventional techniques that can suppose a real break through in biomedical research, and health care. One of the best chances in this direction would come from the development of multipurpose nanometric systems incorporating several physical (magnetic, optical) and biological functionalities in a single unit that could perform simultaneously several operations such as driving, sensing, imaging and therapy. Here, we present a core-shell multifunctional nanoplatform containing magnetic nanoparticles, luminescent centres, anchoring sites for biological active molecules, and potentially a molecular thermometer. The nanoplatform is stable in biological fluids, showed low toxicity, ability for cell internalization, anticoagulation properties, and excellent performance in magnetic resonance imaging and hyperthermia. The magnetic properties of the nanoplatform (magnetic moment, susceptibility, blocking temperature, relaxivity, etc) can be tuned in the whole superparamagnetic range and further by changing the size of the magnetic nanoparticles from 2 to 25 nm. The total particle size can be varied from 30 to 150 nm. The synthesis is based in a polymeric route, and all the components are biocompatible.

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INFLUENCE OF BIOMATERIALS IN THE FORMATION OF POLYURETHANE AND POLYUREA NANOPARTICLES

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Nanoparticles are macromolecular solid colloidal particles of less than 500 nm, in which drugs or other biologically active molecules may be dissolved, entrapped or encapsulated, chemically attached to the polymers or adsorbed to the particle surface. Polyurethanes and polyureas are an important class of polymers widely used in the medical and pharmaceutical fields due to their excellent physical properties and good biocompatibility. In the literature both polymers, had basically been used to achieve microparticles formation with and average size from 10 to 200 ¥ìm. Nevertheless there is a trend towards the preparation of particles in the nanometer range to improve their application as drug delivery systems.
In the design of the nanoparticles for targeting drugs to specific tissues, the development of polymeric shells labelled with hydrophilic or neutral molecules such as polyethylene glycol and lysine is especially noteworthy to avoid the opsonization and subsequent elimination by the reticuloendothelial system.
The aims of this research have been: a) to prepare polyurethane and polyurea nanoparticles by interfacial polycondensation from nano-emulsions with high water concentration (90%wt) selected from the water/ Tween 80/ oil and the water/ Cremophor EL/oil systems and b) to modify the nanoparticle surface with hydrophilic biomaterials (polyethylene glycol 400 and lysine) to avoid endocytic uptake of cell populations belonging to the mononuclear phagocyte system.
To prepare polyurethane and polyurea nanoparticles, a monomer such as isophorone diisocyanate and components with hydroxyl or amine groups are required. O/W nano-emulsions were prepared by the stepwise addition of the aqueous phase (water, PEG 400 aqueous solution or lysine aqueous solution) at 25¨¬C to oil/surfactant mixtures containing isophorone diisocyanate, homogenizing all components under mechanic stirring. These compositions were heated at temperature above than 50¨¬C to obtain nanoparticles. Polymerization reaction was completed after 4 hours and samples were kept at 25¨¬C.
Nanoparticles were characterised by Dynamic Light Scattering, Transmission Electron microscopy (TEM) and Atomic Force Microscopy (AFM) and their stability at 25¨¬C was also studied. Monodisperse nanoparticles with a diameter in the range of 50 to 120 nm were prepared from O/W nano-emulsions. Particle sizes obtained were lower than those described previously in the literature. The smallest nanoparticles were achieved with lysine as biomaterial.

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Predicting the fate of nanocarriers after intravenous injection. B. Naeye1, K. Raemdonck1, H. Deschout1, M. Röding2, M. Rudemo2, K. Braeckmans1 and S.C. De Smedt1
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Interaction with blood proteins is an important parameter when contemplating intravenous injection of nanoscopic gene delivery systems. Injected nanoparticles not only encounter proteins but also a vast population of cells such as erythrocytes, thrombocytes and various leukocytes. Aiming to develop a nanoscopic gene delivery system showing minimal interactions with blood, siRNA loaded dextran nanogels with different charges and PEGylation degrees were incubated with citrated human blood and subsequently measured by flow cytometry. Using fluorescently labeled siRNA and monoclonal antibodies, different cell populations were distinguished and their interactions with different types of nanogels determined. In addition, a new method based on single particle tracking was used to determine the fraction of nanogels bound to cells after incubation with human blood. Results indicate that the charge and not the PEGylation degree of the nanogels is the factor governing the interactions with blood cells. PEGylation on the other hand, was shown to be required to prevent aggregation of nanogels in human plasma. We believe that these newly developed methods can be interesting tools for predicting the effects of drug delivery systems after intravenous injection.

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DEVELOPEMNT AND EVALUATION OF SELF NANOEMULSIFYING SYSTEM OF ETOPOSIDE: IN-VITRO CHARACTERIZATION

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The objective of the present investigation was to develop, evaluate and characterize self-nanoemulsifying drug delivery system (SNEDDS) for improving the delivery of a BCS class IV anticancer agent, etoposide. The solubility of etoposide in oils, surfactants and cosurfactants, was evaluated to identify the components of the nanoemulsion. On the basis of solubility studies, Clove oil was found to provide the highest solubility (216.71mgmL-1) among all medium-chained oils were selected. Efficient self-emulsification was observed for the systems composing of Tween20 as a surfactant and Labrasol as a cosurfactant. Surfactant and cosurfactant (Smix) were mixed in different weight ratios (1:0, 1:1, and 2:1, 3:1, 1:2 and 1:3) and pseudoternary phase diagrams were constructed to identify the nano-emulsification existence zones for the rational design of SNEDDS formulations. SNEDDS formulations were tested for nano-emulsifying properties, and the resultant formulations (SNE1, SNE2, SNE3, SNE4 and SNE5) loaded with etoposide and were further investigated for different in-vitro attributes i.e. thermodynamic stability tests, turbidity, globule size and shape, zeta potential and in-vitro dissolution profile. The SNEDDS yielded nanoemulsions with an average globule size below 100 nm using a Zetasizer 1000 HS (Malvern Instruments, Worcestershire, UK). The SNEDDS was robust to dilution and did not show any phase separation and drug precipitation even after 24 h. TEM photographs of nanoemulsions formulations further conformed the spherical shape of globules. Dissolution rate of etoposide was measured by dialysis bag method using phosphate buffer pH 6.8 as dissolution media. Developed high-performance liquid chromatography method was used to determine drug content in dissolution media at 283 nm. Among the various SNEDDS formulations, SNE3 comprising Clove oil (16.67% v/v) and Smix 2:1 (38.89% v/v) showed maximum drug release when compared to pure drug solution and marketed formulation. These results encourage further development of etoposide SNEDDS as an oral drug delivery system.

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ATTACHMENT OF BIOFUNCTIONALIZED POLYSILICON BARCODES TO THE ZONA PELLUCIDA OF MOUSE EMBRYOS

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Single cell tagging for tracking purposes is gathering increasing attention among the scientific community (1). In fertility centers, tagging of gametes and embryos would be of great interest for sample identification and would help to prevent mismatching errors. Our group has already demonstrated that mouse embryos can be successfully tagged using silicon-based barcodes. Mammalian preimplantation embryos are surrounded by an extracellular glycoprotein coat, the zona pellucida (ZP), which is lost before embryo implantation, a process known as hatching (2). Our first embryo tagging approach was to microinject the barcodes into the perivitelline space of zygotes. The tagged embryos developed normally in culture and could be tracked until the blastocyst stage (3). Here, we present a new approach based on the biofunctionalization of the barcodes to attach them to the outer surface of the ZP. With this approach the direct contact between the barcode and the embryo is avoided and the barcodes would be lost after embryo hatching.

Polysilicon barcodes (10 x 6 x 1 mm) containing 8 rectangular bits were fabricated using silicon microtechnologies (3,4). They were biofunctionalized by self-assembled monolayers (SAMs) with Wheat Germ Agglutinin (WGA), a lectin that binds to specific monosacharides of the ZP glycoproteins. Three different concentrations of WGA (15, 25 and 35 mg/ml) were assayed. As a positive control of biofunctionalization, a protein coupling kit was used to covalently link WGA to carboxylate polystyrene microspheres 6 mm in diameter (Polysciences, Inc.). In vivo fertilized mouse zygotes were rolled over biofunctionalized barcodes or microspheres on a culture dish until 10 barcodes/microspheres were attached to each embryo. To assess biofunctionalization efficiency, embryo viability and retention of barcodes/microspheres during 96 h of culture were determined.

In vitro development of embryos tagged with WGA-microspheres was similar to that of control non-tagged embryos and they kept 96% of the microspheres attached to the ZP during culture. A similar retention rate was achieved with WGA-barcodes when 25 and 35 mg/ml of WGA were used. However, the viability of embryos with WGA-barcodes was lower than the control. It has been reported that free WGA impairs embryo viability, as it can cross the ZP and bind to plasma membrane glycoproteins (5). Our results suggest that unspecific adsorption of WGA molecules probably occurs during biofunctionalization and that these molecules can be released during embryo culture and bind to the plasma membrane. Therefore, our biofunctionalization protocol needs to be further optimized.

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Biostabilized silver nanoparticles based topical antimicrobial gel formulation for the treatment of burns, wounds and diabetic ulcers

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Silver is an effective antimicrobial agent with low toxicity, which is important especially in the treatment of burn wounds where transient bacteremia is prevalent and its fast control is essential. Drugs releasing silver in ionic forms are known to get neutralized in biological fluids and upon long-term use may cause cosmetic abnormality, e.g., argyria and delayed wound healing.

This presentation will give an account of our work on development of an antimicrobial gel formulation containing a new form of silver, viz. biostabilized silver nanoparticles (SNP) in the size range of 7-20 nm synthesized by a proprietary process patented world-wide. The typical minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of SNP against standard reference cultures as well as multidrug-resistant organisms are 0.78-6.25 ìg/mL and 12.5 ìg/mL, respectively. Gram-negative bacteria are killed more effectively (3 log10 decrease in 5-9 h) than Gram positive bacteria (3 log10 decrease in 12 h). SNP also exhibit good antifungal activity (50% inhibition at 75 ìg/mL with antifungal index 55.5% against Aspergillus niger and MIC of 25 ìg/mL against Candida albicans). Interaction of SNP with commonly used antibiotics is synergistic (ceftazidime), additive (streptomycin, kanamycin, ampiclox, polymyxin B) and antagonistic (chloramphenicol). Interestingly, SNP exhibit good anti-inflammatory properties as indicated by concentration-dependent inhibition of marker enzymes (matrix metalloproteinase 2 and 9). The post agent effect (a parameter measuring the length of time for which bacterial growth remains suppressed following brief exposure to the antimicrobial agent) varies with the type of organism (e.g., 10.5 h for P. aeruginosa, 1.3 h for Staphylococcus sp. and 1.6 h for Candida albicans). The antibacterial spectrum of the gel formulation containing SNP (S-gel) is found to be superior to commercial formulations of silver sulfadiazine, especially against multi drug resistant clinical isolates. As part of toxicity studies, localization of SNP in Hep G2 cell line, cell viability, biochemical effects and apoptotic/necrotic potential have been assessed. It is found that SNP get localized in the mitochondria and have an IC50 value of 251 ìg/mL. Even though they elicit an oxidative stress, cellular antioxidant systems (reduced glutathione content, superoxide dismutase, catalase) get triggered and prevent oxidative damage. Further, SNP induce apoptosis at concentrations up to 250 ìg/mL, which could favor scarless wound healing. Acute dermal toxicity studies on SNP gel formulation (S-gel) in Sprague-Dawley rats showed complete safety for topical application.

Phase III multicentre clinical trials of S-gel have been successfully conducted for indications such as burns, wounds and diabetic ulcers. The Drug Controller General of India (DCGI) has recently approved it as a new drug.

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FITC labeling of proteins increase binding capacity of chitosan nanoparticles
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Chitosan is a biodegradable and biocompatible natural polycation which has high affinity for epithelial cells. It is widely used for nanoparticles (NPs) production which can be used as a carrier for delivery of drugs or peptide/protein vaccines. However, positively charged chitosan NPs form complexes mostly with negatively charged molecules and this limits their usage. This work aimed to study complex formation between chitosan NPs and positively charged or neutral proteins. To increase negative charge of proteins they proteins were modified by negatively charged FITC molecules.

NPs were produced from N-hexanoyl-chitosan by ionotropic gelation with tripolyphosphates. Earlier we showed that N-hexanoyl-chitosan NPs with 10% N-hexanoyl group content are stable and have average size 500 nm. Acidic protein bovine serum albumin (BSA, total charge -14), neutral human superoxide dismutase (SOD, charge -4), sperm whale myoglobin (SWM, +2), and basic protein lysozyme (Lys, charge +12) were labeled with FITC. Complex formation was analyzed by Bradford assay in residual supernatants and by acrylamide gel-electrophoresis of chitosan NPs/protein sediment.

Earlier we have shown that protein loading capacity of chitosan NPs depends directly on protein charge while the stability of such complexes – on ionic content of the buffer. To increase negative charge of proteins we decided to introduce FITC molecules which contain carboxyl groups. We have shown that binding of FITC-labeled proteins increases significantly not only of neutral and basic proteins but also of acidic protein BSA when compared to unlabeled proteins. Sorption efficacy for BSA increased 1.5-1.9 times and reached 85%. Binding of SOD, SWM and Lys from undetectable level increased several folds and reached 50%, 39%, and 65% accordingly.

Thus, modification of proteins by small negatively charged molecules like FITC which do not interfere with protein function (as it is shown for FITC) can be used to increase loading efficacy of proteins or peptides to chitosan NPs. This can be applied not only to neutral or positively charged proteins and peptides but also to acidic proteins.

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Title: Nanoscale delivery of micronutrients and nutraceuticals: Differences and similarities with drug delivery

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The delivery of functional ingredients, similarly to drug delivery, brings enormous challenge for the food industry. For example, the incorporation of micronutrients and nutraceuticals can compromise the product functionality. The issues often encountered are related to solubility, taste, and stability of the functional ingredient or unwanted changes in the product stability, appearance, texture, and taste due to interactions with other ingredients. As opposite to drug formulation, in food the emphasis is mainly on maintaining the overall functionality of the products rather that on the active ingredient alone. The formulation of lyophobic bioactive molecules with high melting temperature, similarly to many drugs, can also be problematic due to their limited solubility and bioaccessability. To overcome these issues, a novel approaches based on colloidal delivery systems have been developed. Functional colloidal carries are designed to bring the necessary balance between soluble and insoluble state of the functional ingredient without compromising the final product. In this talk, the approach based on the use of colloidal delivery systems in functional food design will be illustrated. The basic concepts for design and application of colloidal delivery systems will be exemplified. Finally, the differences and similarities with drug delivery related issues will be discussed.

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Title: Polymersome penetration into human skin
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The aim of our research is to develop and characterise a novel drug delivery solution based on flexible nano-sized polymeric vesicles called polymersomes. These polymersomes have been shown to penetrate into the deep layers of human skin and to be capable of delivering therapeutic payloads directly into the cell cytoplasm. They are formed from high molecular weight amphiphilic pH sensitive block copolymers ((poly(2-(methacryloyloxy)ethyl-phosphorylcholine)-co–poly(2-(diisopropylamino) ethyl methacrylate) (PMPC–PDPA),) that self-assemble into vesicles when exposed to an aqueous environment. Skin is a highly stratified and impermeable tissue with specific size, charge, hydrophilic and lipophilic restraints on the kinds of molecule that can diffuse across it. By using a perfusion system developed in our laboratory we've demonstrated that polymersomes are capable of crossing this biological barrier successfully. This perfusion system is divided into a top-donor and a bottom-acceptor chamber. The tissue sample is placed between these two chambers allowing us to see how much of the polymersome solution placed in the donor chamber can diffuse into the acceptor chamber.
Polymersome diffusion was performed across ex-vivo skin, tissue engineered skin models and synthetic porous polycarbonate membranes with an average pore size of 50 nm. Results show that polymersome diffusion is strongly dependant on concentration, flow and the ratio between vesicle and membrane pore size. With higher concentration gradients and flow rates diffusion is increased, confirming dependence on the osmotic gradient across the barrier. We prepared 400 nm and 200 nm diameter sized vesicles and placed them on top of 50 nm pore sized membranes inside the perfusion system. We reproduced the same ambient conditions for each experiment and we found that very large vesicles diffused more easily through small pores (approximately 70-90% of the initial polymersome solution diffused across the membrane) compared to vesicles with approximately the same size as the pore (diffusion was around 20-30%). Most importantly we found that polymersomes maintained structural stability and did not defragment during diffusion (as shown by Dynamic-light-scattering analysis, DLS), thereby proving that they possess mechanical properties that allow them to deform and squeeze in the gaps between the tightly packed dead cells of the top skin layers. This was further confirmed by measuring their ability to carry model molecules such as dextran across the barrier without significant loss. These promising results support using this novel delivery system with potential applications spanning from cosmetics, to dermatology to vaccine technology.

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Analyses of various biomolecular interactions using spinning-multilayered disk biosensor

A spinning-disk biosensor utilizing optical interference of reflected light from a multilayered structure, consisting of dielectric, metal, and optical phase-change thin films, is shown to have the potential to monitor various interactions on its surface. We refer to this platform as a BioDVD, since it utilizes the optical system of a digital versatile disk (DVD) to measure changes in reflected light intensity. In the past, several technologies are currently available for the analysis of biomolecular interactions with high sensitivity and efficiency. However, these instruments are invariably expensive and, thus, are not suitable for bedside analyses. To circumvent this issue, we have previously reported a BioDVD platform that allowed us to use a DVD mechanism to monitor various biomolecular interactions [Gopinath et al., 2008, ACS Nano 2, 1885-1895; Anal.Chem. 2009, 81, 4963-4970]. In order to improve the sensitivity of the BioDVD platform, we have carried out several studies to address the importance of the thickness of multilayered disk structure and also improving data acquisition and analysis for different biomolecular interactions that includes protein-protein, RNA-protein and RNA-small ligands. Some of these recent developments will be presented at the conference.

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Biofunctionalization of micronanotools to tag living cells

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Cell tracking is an emergent area in nanobiotechnology due to its interest for studying individual cells or to distinguish populations of cells in vivo [1]. Our approach is based on tag externalization and relies on using appropriate micronanotools with the idea to attach them to the cell membrane of living cells.
The design of these micronanotools considers three main components: a barcode functionalized through a linker to a biomolecule performing the tagging function. Barcodes of different materials, mainly gold and polysilicon, were obtained using fabrication technologies according to a previously reported methodology [2]. The biofunctionalization process has been studied in order to find the optimal conditions to form protein self-assembled monolayers (SAMs) on different material surfaces. In particular, several lectins have been used due to its capacity to recognize some specific carbohydrates which are expressed on the embryos zona pellucida. The influence of the chemical nature of the components of these micronanotools, such as the constitution of the SAMs with different terminal groups to study the better link to the biomolecule, in the tagging experiments will be reported. The SAMs have been characterized by different techniques such as contact angle measurements, TOF-SIMS, LDI-TOF MS and AFM, and also fluorescence measurements for the biofunctionalized barcodes. Ultimately, the barcode adhesion was tested on living cells.

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Anionic drug nanocarriers from bis-imidazolium amphiphiles

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Nanotechnology, in a wider sense, and nanomedicine in particular, are two fields that have experienced a growth in recent years, spawning various research disciplines.1 One of them is the research and development for new molecules that can be used to design nanocarriers (such as nanoparticles for drug delivery) and to develop diagnostic and/or biosensing tools (such as DNA microarrays for gene recognition). These molecules can be combined with already well-known materials, such as gold. Gold is widely used to assemble nanoparticles for targeted therapy and biodiagnostic tools, for its physical and chemical characteristics, and also as matrix in the design of biosensors, whether in the form of immobilized nanoparticles or as a functionalized surface.

In this work, we present a novel bis-imidazolium amphiphile able to behave as an anionophore2 and to interact with gold.3 In solution, this interaction occurs with concomitant stabilization of gold, producing nanoparticles with a size around 5-8 nm. Therefore, the bis-imidazolium plays a triple role as a synthesis promoter, a stabilizer of the gold nanoparticles and also, as an anion receptor, making these nanoparticles appear as suitable carriers for negatively charged drugs.

The ability to incorporate a model drug yielding a negatively charged carboxylic group will be adressed, and the release from the nanoparticles reported. The work in progress also includes the assembly of a monolayer to functionalize gold surfaces, and its characterisation.

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The Nanomechanical Properties of the Endothelial Glycocalyx in ex vivo Arteries.
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The endothelial glycocalyx (eGC) is a several hundreds of nanometres thick layer that covers the luminal side of blood vessels. This fragile structure is an anionic biopolymer that mainly consists of proteoglycans and glycoproteins. The eGC contributes to the barrier function of the endothelium, acts as a regulatory microdomain for many processes at the membrane surface and is also a mechanosensor which is involved in fluid shear stress response of endothelial cells. Recently, the eGC was recognized as a structure that is crucial for maintaining normal vascular function. Despite the importance of the eGC little is known about its mechanical properties.
To gain more insight into the mechanics of eGC, nanoindentation measurements were performed with atomic force microscopy (AFM) on living endothelial tissue from murine ex vivo arteries. The unique benefits of AFM are real-time stiffness measurements under physiological conditions. Force measurements were performed using soft cantilevers (spring constant = 18 pN/nm) with spherical tips (sphere diameter = 1 µm) and maximal loading forces in the range of 300 pN. Analysis of the data revealed a soft layer on the surface of endothelial cells with a stiffness of about 0.4 pN/nm and a height of about 70 nm. As a control experiment, application of 1 U/ml heparinase, an enzyme that selectively digests heparane sulphate residues of the glycocalyx, was applied. Heparinase treatment led to the reduction of this soft surface layer in height and stiffness to about 50 nm and 0.2 pN/nm respectively. This indicates that this layer is most likely the endothelial glycocalyx.
In conclusion, a method was established to characterize the mechanical properties of the eGC at the nanoscale. This technique will provide better understanding of the eGC regulatory processes e.g. mechanotransduction of fluid shear stress and endothelial barrier function. This technical approach should allow real-time studies of drug interaction with endothelial glycocalyx.

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Predicting the fate of nanocarriers after intravenous injection. B. Naeye1, K. Raemdonck1, H. Deschout1, M. Röding2, M. Rudemo2, K. Braeckmans1 and S.C. De Smedt1
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Interaction with blood proteins is an important parameter when contemplating intravenous injection of nanoscopic gene delivery systems. Injected nanoparticles not only encounter proteins but also a vast population of cells such as erythrocytes, thrombocytes and various leukocytes. Aiming to develop a nanoscopic gene delivery system showing minimal interactions with blood, siRNA loaded dextran nanogels with different charges and PEGylation degrees were incubated with citrated human blood and subsequently measured by flow cytometry. Using fluorescently labeled siRNA and monoclonal antibodies, different cell populations were distinguished and their interactions with different types of nanogels determined. In addition, a new method based on single particle tracking was used to determine the fraction of nanogels bound to cells after incubation with human blood. Results indicate that the charge and not the PEGylation degree of the nanogels is the factor governing the interactions with blood cells. PEGylation on the other hand, was shown to be required to prevent aggregation of nanogels in human plasma. We believe that these newly developed methods can be interesting tools for predicting the effects of drug delivery systems after intravenous injection.

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Anionic drug nanocarriers from bis-imidazolium amphiphiles
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Nanotechnology, in a wider sense, and nanomedicine in particular, are two fields that have experienced a growth in recent years, spawning various research disciplines1. One of them is the research and development for new molecules that can be used to design nanocarriers (such as nanoparticles for drug delivery) and to develop diagnostic and/or biosensing tools (such as DNA microarrays for gene recognition). These molecules can be combined with already well-known materials, such as gold. Gold is widely used to assemble nanoparticles for targeted therapy and biodiagnostic tools, for its physical and chemical characteristics, and also as matrix in the design of biosensors, whether in the form of immobilized nanoparticles or as a functionalized surface.
In this work, we present a novel bis-imidazolium amphiphile able to behave as an anionophore2 and to interact with gold3. In solution, this interaction occurs with concomitant stabilization of gold, producing nanoparticles with a size around 5-8 nm. Therefore, the bis-imidazolium plays a triple role as a synthesis promoter, a stabilizer of the gold nanoparticles and also, as an anion receptor, making these nanoparticles appear as suitable carriers for negatively charged drugs.
The ability to incorporate a model drug yielding a negatively charged carboxylic group will be adressed, and the release from the nanoparticles reported. The work in progress also includes the assembly of a monolayer to functionalize gold surfaces, and its characterisation.

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Magnetic-silica nanospheres. Application as contrast agents for MRI and as drug delivery carriers

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We will report on a convenient method for the synthesis of magnetic silica nanospheres as well as their evaluation as contrast agents for MRI and as drug delivery carriers.

Fabrication of the magnetic silica nanospheres was done in a straight forward one-pot method combining sol-gel chemistry and supercritical fluids technology [1]. Briefly, pre-formed iron oxide superparamagnetic nanoparticles stabilized in hexane are dispersed in a sol containing a silicon alkoxide, water and acetone as the solvent. The precursor solution is introduced in an autoclave and pressure and temperature are raised over the supercritical conditions of the CO2/acetone mixture allowing the silicon alkoxide to hydrolyze and condensate forming the silica shell around the iron oxide particles. The composite gel nanoparticles get dried as the solvent is extracted at supercritical conditions. Resulting nanospheres present a very narrow particle size distribution with sizes of the order of 100 nm. Each nanosphere consists of a magnetic multicore of non-contacting iron oxide nanoparticles surrounded by a microporous silica shell. Nanospheres are superparamagnetic at RT presenting an enhanced magnetization compared to the initial preformed iron oxide nanoparticles. Their size can be tuned by controlling the reaction conditions. Some advantages of the method are short reaction times, purity of the product and potentiality of the method to be scaled up. Cytotoxicity of the composite will be also reported.

Regarding their potential use as MRI contrast agents, it is to be highlighted that the composite nanospheres present very high transversal relaxivity values (326 s-1mMFe-1 at 20 MHz, 37ºC) which can be linearly correlated with the magnetic moment of the composite particles [2]. Finally, the use of supercritical carbon dioxide (scCO2) to impregnate a therapeutic agent (triflusal) in such nanospheres will be also presented.

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We are grateful to Prof. R. Weissleder at the Center for Molecular Imaging Research, Massachusetts General Hospital for the relaxivity and cytotoxic measurements performed by E.R. during the post-doc stay in his group.

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In vivo study of a nanoparticle based formulation for combined chemo-immunotherapy against cancer

Resistance to conventional chemotherapy in patients with advanced tumors has prompted the need of alternative cancer therapies. Immunotherapy for cancer is an emerging effective approach which employs stimulation of the immune system to eradicate cancer cells. If immunotherapeutic approach is synergistically applied along with chemotherapy, the residual drug resistant tumor cell population can be targeted and can have synergistic effect. A nanoparticle based system encapsulating a cytotoxic drug and an immunostimulant not only offers the aforesaid benefits but also have the advantage of tumor targeted delivery to minimize the unwanted effects of the both.

The prime objective of this study was to develop poly (lactic-co-glycolic acid) (PLGA) nanoparticles (NP) encapsulating Paclitaxel (PTX) and a non-toxic derivative of lipopolysaccharide (LPS). PTX is a promising chemotherapeutic agent, but due to its water insolubility, it cannot be used up to its potential in clinical setup. LPS is a well established TLR4 agonist and a potent immunostimulant, but is toxic. Non toxic derivatives of LPS retain its immunostimulatory activity, but the toxicity reduces many folds. The co-administration of both as NP dosage form offers a localized chemo-immunotherapeutic response as well as potential pharmacokinetic advantages.

Particles were prepared by single emulsion method (o/w) and the size was found to be in the range of 200-300nm, homogeneous and had negative charge which is desirable for iv administration. From Transmission Electron Microscopy, the NPs were found to be spherical and uniform in shape. The release behavior of PTX from the NPs exhibited biphasic pattern characterized by an initial burst followed by slow continuous release. In vitro direct cytotoxicity of NPs on B16-F10 melanoma was found to be comparable with commercial Paclitaxel. In-vitro cell cycle arrest study also showed the same pattern. In vitro delivery of the NPs was evaluated using coumarin loaded fluorescent NPs and it was found that the particles readily deliver the encapsulated drug to the cancer cells. In-vitro stimulation of splenocytes with the NPs showed induction of pro-inflammatroy cytokines like TNF-A;, IFN-γ, IL-12. In vivo tumor regression study in mouse model confirmed the synergistic chemo-immunotherapeutic activity of the NP dosage form. Significant increase in the number and activation status of infiltrating lymphocytes (TIL) was observed in the NP treated group as compared to the control. These results provide evidence that this NP dosage form might have potential as a chemo-immunotherapeutic formulation against cancer.

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PGA based conjugates in antiangiogenic cancer therapy

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Paclitaxel is a well know cytotoxic agent that is frequently used in anticancer therapies. At low concentrations, this drug also shows antiangiogenic activity. However, its use is limited by some severe side-effects.
In this study, a new polymeric conjugate of paclitaxel has been developed to improve the therapeutic index of this hydrophobic molecule. In order to target selectively the neovasculature around the tumor tissue, RGD residues has also successfully been introduced in the nanostructure. This small oligopeptide is reported to be able to recognize the alphav-beta3 integrins, which are only expressed in new blood vessels, resulting in a clear antiangiogenic activity. This way, we have developed a new system for combination therapy.
The results on the characterization and some of the biological activity of this conjugate will be discussed in the present piece of work.

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IOPROMIDE AND Tc-99m LOADED NANO-SIZED TUMOR TARGETED LIPOSOMES FOR TUMOR IMAGING BY SPECT/CT

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With the developing technology and improvements in the computer engineering, science enable refinements and the generation of novel imaging modalities that combine imaging instruments that are used in Nuclear Medicine provided metabolic and Radiology provided anatomical information. Depending on these developments, there is an essential need for the novel contrast agents that specifically delivers to the target, gives sufficient signal/contrast intensity and less target/background contrast ratio for obtaining better images with less time consuming. Liposomes have been using for a long time to develop contrast agents for different imaging modalities because of their properties such as biocompatibility, bioavailability, loading capacity of different contrast agents and ligands in the aqueous core and/or on the lipid bilayer.

When visualization of small pathological areas, such as tumors, is required, the local contrast agent concentration in these areas becomes crucial. PEGylated liposomes have been suggested to increase the local concentration and to prolong the plasma half-life of contrast agents. To increase the accumulation of drug-loaded liposomes (including liposomes loaded with contrast agents) in various targets including tumors, the use of specifically targeted liposomes was also suggested. For this purpose, plain or long-circulating liposomes are additionally modified with target-specific ligands, usually monoclonal components against characteristic component of the pathological tissue (1,2). Earlier, family of natural antinuclear autoantibodies with nucleosome(NS)-restricted specificity capable of specific recognition of a broad variety of live cancer cells via cancer cell surface-bound NS, which are released by apoptotically dying neighboring cancer cells were identified (3).

Here, we demonstrate that Iopromide and Tc-99m loaded liposomes can be additionally modified with the monoclonal anticancer antibody 2C5 (mAb 2C5) possessing the nucleosome-restricted specificity via the PEG spacer. Liposome-bound antibody immunoliposomes specifically recognize various cancer cells in vitro and target an increased amount of contrast agent/radionuclide to their surface compared to antibody-free liposomes.

The results showed that mAb 2C5 modified PEGylated liposomes containing Iopromide specifically bind to the cancer cells and binding capacity of immunoliposomes are 4-5 times more than control non-modified liposomes in vitro. The interaction of Iopromide containing PEG-Liposomes with cancer cells were visualized by fluorescent microscopy

As conclusion, Iopromide and Tc-99m loaded cancer cell targeted immunoliposomes may represent promising agents for enhanced tumor imaging by SPECT/CT.

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Stochastic nanoparticle dose delivery and dilution in proliferating cell populations
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Nanomedicine endeavours to treat disease at the cellular level, directly targeting unhealthy cells using nanoparticle delivery vehicles with well defined cargos of drug molecules. Often the natural cellular uptake mechanism of endocytosis is used to deliver nanoparticles into cells via attachment of ligand-decorated particles to cell surface receptors. Once internalised they can release therapeutic agents, activate prodrugs through reduction within the acidic, endosomal environment or remain as multi-generational reporters. Whilst there has been much recent progress in the creation and delivery of nanomedicines a complete understanding of nanoparticle pharmacokinetics in proliferating cells has yet to be elucidated. What role does endocytosis play in determining cell to cell dose variation and how does mitosis affect the heterogeneity of nanoparticle inheritance? Here we show that nanoparticle uptake into a U2-OS tumour cell line and the subsequent dose dilution by cell mitosis is stochastic, being the outcome of random Bernoulli trials. A statistical analysis of nanoparticle-loaded endosomes indicates that particle capture is described by an over-dispersed Poisson distribution consistent with heterogeneous adsorption and internalisation processes. The subsequent partitioning of nanoparticles in cell division is both stochastic and asymmetric; following a binomial distribution with a mean probability of 0.62-0.72. These results have ramifications for the implementation of medicine at the nanoscale, suggesting that the cellular targeting of nanoparticles is inherently imprecise due to the randomness of nature at the molecular level.

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Potential of using nanomedine to improve the treatment of poverty related diseases
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Poverty-related diseases (PRDs) are the major cause and consequence of considerable poverty in Developing countries, particularly Sub-Saharan Africa. The annual global death toll of HIV/AIDS, malaria and tuberculosis (TB) approaches 6 million people. Fighting these diseases remains one of the most effective ways to alleviate poverty and promote economic progress in these countries.

Although effective therapeutic regimens against these diseases are available, treatment failure due to poor adherence, which in turn leads to the emergence of drug resistant strains remain a challenge. For TB, Directly Observed Treatment (DOTS) is the WHO internationally recommended strategy to ensure adherence to TB treatment. In South Africa DOTs success is at 55%, which is far from the expected 85% target by WHO. Other shortfalls of current therapies include high dosage and dose frequency due to poor bioavailability, which lead to long duration of treatment and negative side effects; hence poorer treatment outcomes and increased cost of treatment. We propose the use of nanomedicine to address the current shortfalls of PRD therapies, using TB as a case study.

To date, the CSIR drug delivery platform has successfully nano encapsulated all four first line anti-TB drugs (i.e Rifampicin (RIF), Isoniazid (INH), Ethambutol (ETB), and Pyrazinamide (PZA)), in polymeric nanoparticles with an encapsulation efficiency above 50% in particles of 250-400nm, using a novel multiple emulsion spray-drying technique. A PCT patent on the method of encapsulation has already been filed. It was observed from in vitro release assays, that the drugs were released in a slow manner over a period of several days. Furthermore, intracellular drug delivery studies were performed in two human cell lines where it was demonstrated that the particles are taken up by the cells and delivered through the phagosomes into the cytoplasm. Subsequently, the bacterial growth index in H37RV infected THP-1 cells treated with encapsulated rifampicin was reduced significantly when compared to that of cells treated with free rifampicin. Extracellular bacteria were also killed by the encapsulated drug over a period of time. Drug release was observed in vivo over a period of 6 days in laca mice and the MIC (minimum inhibitory concentration) for RIF and INH was maintained over this period. The extent of tissue biodistribution and retention post oral administration of PLGA particles to Balb/C mice was analysed for 7 days after which the particles were still detected in the brain, heart, kidney, liver, lungs and spleen. It was reported that although these particles persist in the mice for a number of days, no toxicities were observed (Semete et al., 2010). Furthermore, a pre-clinical study in TB mice models demonstrated that the encapsulated drugs, administered once weekly, resulted in a comparative reduction in the colony forming unit (cfu), i.e bacterial burden when compared to the free drugs that were administered once daily. We have further actively targeted TB infected macrophages with nanoparticles that are functionalised with aptamers against the target protein

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Kelvin Probe Force Microscopy Study of the (001) GaAs Surface Decorated with Human Carcinoma Cells

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Optical and electronic properties of III-V semiconductor quantum well (QW) and quantum dot (QD) microstructures are attractive for building biosensing devices. They can be used to detect miniscule perturbations of the semiconductor surface induced by selectively trapped electrically charged biomolecules. Viruses, bacteria and biological cells provide attractive interfaces with III-V QW and QD microstructures to be investigated in that context.

Here we discuss the results of immobilization of human carcinoma cells A-549 on the surface of GaAs (001). As a reference, we have also investigated the immobilization process of A-549 on Au (111) surfaces. The immobilization was achieved, first, by depositing a self-assembled monolayer (SAM) of 2 mM16-Mercaptohexdecanoic acid. Second, the samples with SAMs were immersed in an aqueous solution of 0.4 M EDC and 0.1 M N-hydroxysuccinimide (NHS) in order to activate the COOH groups suitable for the attachment of fibronectin. The exposure of such prepared surfaces to 104 - 105 A-549 cell culture/mL solution enabled us to immobilize live cells. Atomic force microscopy (AFM) measurements were carried out for samples with cells fixed and permeabilized using formaldehyde and triton-X100, respectively. The results indicate that it is possible to efficiently decorate surfaces of functionalized both Au (111) and GaAs (001) by the direct cell cultivation process. The distribution of the electric charge in the cell was investigated using the Kelvin Probe Force Microscopy (KPFM) technique. This enabled us to conclude that the charge distributions in live and dead cells are comparable macroscopically. However, a detailed analysis of the electrical properties of the cell membrane is required to investigate this process microscopically.

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Preparation and Cellular Characterization of Stealth Immunoliposomes Targeted against HER-2 in Breast Tumor Cells

Stealth liposomes, nano-sized vesicular bilayer membranes comprising PEGylated phospholipids, are regarded as carriers for prolonged drug delivery and passive targeting to tumor cells. To promote selectivity of the carrier, mAb-tagged liposomes named immunoliposomes are developed; however, antibody conjugation might compromise in-vivo fate of the carrier. Therefore, to actively target antiapoptotic HER-2 receptor in breast cancer cells, we aimed to prepare trasuzumab-conjugated Stealth liposomes that benefit both antiapoptotic and active targeting characteristics of trastuzumab, and phospholipid PEGylation for prolonged blood circulation. Moreover, Rhodamine-PE is included for bio-imaging purposes.

Stealth liposomes containing DPPC / DPPG / PEG-PE / cholesterol were prepared at different mole ratios and sized by extrusion. Rhodamine-PE 0.5% mole was added to the lipid mix if applicable. PEG-PE-Maleimide micelles were prepared by sonication of methanolic dispersion (1 mM) for 5 min followed by dialysis against HBS (pH=6.5). Trastuzumab, mAB against HER-2, was activated by Traut's reagent for 1 hour and coupled to PEG-PE micelles through disulfide linkage. The degree of thiolation was determined by GSH and Ellman's reagent. Consequently, immunoliposomes were prepared by post insertion method. Particle size distribution and mAb conjugation yield were determined by laser light scattering and HPLC-based gel permeation chromatography, followed by Bradford reagent protein quantification.

Stealth liposomes were homogeneous 100-110 nm particles that demonstrated uni-lamellar vesicular structure as determined by Epifluorescent microscopy of Rhodamine-PE labeled phospholipid. SDS-PAGE and HPLC-SEC of Trastuzumab solution 0.01% showed a monodisperse 150kDa protein. PEG-PE micelles were prepared at concentration about 100-fold above CMC (<10 ìM) represented colloidal dispersion with size ranging from 5-50 nm. Following micelle conjugation to activated trastuzumab and immunoliposome preparation, the specific binding capacity of the carrier is going to be checked in HER2+ (SK-BR-3) and HER2– (MCF-7) breast cancer cells by fluorescent techniques.

As a conclusion, the bioconjugates of Stealth nanoliposomes expected to be useful for active targeted cellular delivery of their cargo such as biopharmaceuticals and for simultaneous diagnostic purposes.

Keywords: Immunoliposome, Trastuzumab, Bioconjugation

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Preparation and characterisation of hyaluronate-based nanoparticulate carriers intended for delivery of biomolecules

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Due to the many disadvantages of injections alternative methods to efficiently deliver biomolecules are being sought. One of the methods that may enhance bioavailability of such molecules as well as entrap and protect the delicate cargo against chemical and physical degradation is complexation with polymers to form colloidal in size entities, polymeric nanoparticles (NPs). The aim of this work was to optimise the complex NPs based on two biodegradable polymers, hyaluronic acid (HA) and chitosan, to obtain nanoparticles suitable for encapsulation of macromolecules and varying in charge from negative to positive.

The nanoparticles were prepared by the formation of polyelectrolyte complexes. Different molecular weight HA obtained by depolymerisation by sonication and a range of chitosan salts with different molecular weights were tested (chloride: CL113 50-150 kDa, CL213 150-400 kDa and glutamate: G113 50-200 kDa, G213 200-600 kDa).

When the HA solution was not sonicated prior to the formation of particulates, micron-sized, sedimenting aggregates were formed. No sedimentation was observed when the HA solution was treated with ultrasounds for at least 30 minutes. The molecular weight of HA was reduced from 1726+/-50 kDa to 176+/-4 kDa after 6 hours of sonication. Also, the viscosity of this sonicated HA solution was dramatically reduced compared to the non-sonicated HA (0.98+/-0.02 and 15.49+/-0.81 mPa*s, respectively). Infrared analysis was consistent with no chemical changes in the HA structure. Aggregates were also formed when the high molecular weight salts of chitosan were used (CL213 and G213) or when the charge mixing ratio of polymers was close to 1. Positively and negatively charged nanoparticles were obtained depending on the mixing ratio of the polymers with chitosan glutamate NPs more negatively charged compared to the chitosan chloride-based NPs. The smallest NPs were formed when HA was sonicated for 6h (HA/CL113, size: 149+/-11 nm; zeta potential: -33.9+/-1.5 mV). NPs slightly greater in size were obtained with HA sonicated for 2h, using the weight mixing ratio of 2.5 (HA/CL113, size: 172+/-26 nm, zeta potential: -35.7+/-2.0 mV).

Overall, the nanoparticles obtained can be considered as promising carriers for formulating biomolecules due to their favourable micromeritic and biopharmaceutical properties.

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Use of (albumin) lyophilisomes in nanomedicine

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Drug targeting capsules are carrier materials designed to improve the biodistribution of (chemo)therapeutics. A large number of systems has been developed. The preparation of capsules is generally driven by the amphiphilic nature of the building blocks. Previously, our group described a new type of nano/microcapsule, named 'lyophilisomes', which can be prepared using a variety of molecules and is not driven by amphilicity. Lyophilisomes can be prepared by a three step procedure comprising fast freezing (microphase separation), annealing (structural rearrangement) and lyophilization (creating of a lumen). This procedure resulted in nanocapsules up to 3000 nm. Transmission electron microscopy verified that capsules indeed contained a lumen. In this study, lyophilisomes were prepared from bovine serum albumin.

First, a subset of smaller lyophilisomes was isolated, since small particles can passively extravagate and accumulate in tumors because of the leaky vessels. Here we describe a method to obtain lyophilisomes in the nanometer range using flow cytometry. To ascertain that lyophilisomes are detectable with flow cytometry, FITC-positive albumin lyophilisomes were used. First, a gate was set around the small structures to remove larger lyophilisomes as well as sheet-like structures.. This procedure resulted in a lyophilisome population ranging in size from approximately 100-1000 nm as detected by scanning electron microscopy.

To probe the possibility to use lyophilisomes for tumor targeting, we investigated whether they could be loaded with the anti-tumor drug doxorubicin. After a pre-incubation with ammonium sulphate followed by an incubation in doxorubicin, the drug could indeed be entrapped in lyophilisomes as analysed by confocal microscopy.

In conclusion, a new type of nanocapsules (lyophilisomes) can be sized and loaded with tumor drugs.

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Antileishmanial and antifungal activity of amphotericin B nanoformulations

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Purpose The aim of present study was to evaluate the in vitro antileishmanial and antifungal activity of different amphotericin B (AmB) nanoformulations. This investigation focuses on AmB-loaded polymeric nanoparticles (NPs) composed of poly(D,L-lactide-co-glycolide) (PLGA) and nanosuspensions.
Methods The nanoformulations were prepared by means of a nanoprecipitation technique using polyvinyl alcohol as stabilizer. AmB was entrapped into the PLGA NPs at an initial drug loading of 10% or 20% w/w to PLGA. The mean particle size Zave of the nanoparticles was determined using Photon Correlation Spectroscopy. Mannitol was used as cryoprotectant during freeze-drying. Standard in vitro sensitivity tests were performed on mouse peritoneal macrophages infected with Leishmania infantum ex vivo amastigotes, extracellular L. infantum promastigotes and the fungal species Candida albicans, Aspergillus fumigatus and Trichophyton rubrum. EC50s of nanoformulations are expressed as equivalent concentration of AmB.
Results and conclusions Zave of the obtained PLGA NPs depended significantly (p≤0.001) on the amount of co-solvent DMSO used during production. Particles prepared with the combination DMSO/acetone (1:1) were in the size range of 200-254 nm, whereas those prepared with solely DMSO in the range 100-138 nm. The average entrapment efficiency of the AmB-loaded NPs was 58.5±4.6% at 10% w/w initial drug loading vs. 63.3±1.3% at 20% w/w initial drug loading (p≤0.001). The AmB nanosuspension had a mean particle size of 152.2±1.0 nm (PI 0.36) and a much higher drug loading of 35.8±0.1 µg/mg in comparison to the polymeric NPs with 5.3±0.1 µg/mg at 20% w/w initial AmB loading. The EC50 of the different AmB-loaded PLGA NPs on intracellular amastigotes were in the range 0.07-0.15 µg/ml vs. 0.17±0.03 µg/ml for AmB and 0.15±0.004 µg/ml for the nanosuspension, however there were no significant differences between groups (p>0.05). In contrast, the PLGA formulations were more effective against the extracellular promastigotes than the free drug. The mean EC50 of the AmB-loaded polymeric NPs (0.08±0.004 µg/ml) was significantly lower than that of AmB (0.15±0.003 µg/ml) (p≤0.001). There were no significant differences between the different polymeric formulations concerning antileishmanial activity. All AmB nanoformulations had a significantly higher (p≤0.001) in vitro activity on the tested fungal species than the reference drug AmB. A distinction between the antifungal activity of AmB-loaded PLGA NPs and AmB nanosuspension could only be made for T. rubrum. The PLGA formulations were more effective (0.11±0.04 µg/ml) than the AmB nanosuspension (0.29±0.0 µg/ml) (p =0.04), which in their turn were more effective than free AmB (1.17±0.0 µg/ml) (p≤0.001).

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Gene Silencing Activity of siRNA Polyplexes Based on Thiolated N,N,N-trimethylated Chitosan

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N,N,N-trimethylated chitosan (TMC) is a biodegradable polymer emerging as a promising non-viral vector for nucleic acid and protein delivery. In the present study, we investigated whether introduction of thiol groups in TMC enhances the extra-cellular stability of the complexes based on this polymer and promotes the intra-cellular release of siRNA. The gene silencing activity and the cellular cytotoxicity of polyplexes based on thiolated TMC were compared with those based on the non-thiolated counterpart and the regularly used lipidic transfection agent Lipofectamine.

Incubation of H1299 human lung cancer cells expressing firefly luciferase with siRNA/thiolated TMC polyplexes resulted in 60-80 % gene silencing activity, whereas polyplexes based on non-thiolated TMC and lipofectamine showed less silencing (40 and 60 %, respectively). Importantly, the TMC-SH polyplexes retained their silencing activity in the presence of hyaluronic acid, while non-thiolated TMC polyplexes hardly showed any silencing activity, demonstrating their stability against competing anionic macromolecules. Under the experimental conditions tested, the cytotoxicity of the thiolated and non-thiolated siRNA complexes was lower than those based on Lipofectamine. Given the good extra-cellular stability and good silencing activity, it is concluded that polyplexes based on TMC-SH are attractive systems for further in vivo evaluations.

Keywords: thiolated TMC, siRNA, delivery, gene silencing, polyplex stability, cytotoxicity, hyaluronic acid

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A STRATEGIC WATCH ON NANOMEDICINE PERFOMED BY A NETWORK OF SCIENTIFIC EXPERT AT THE OBSERVATORY FOR MICRO AND NANOTECHNOLOGIES, ANNE-MARIE VILLARD, CNRS - OMNT (FRANCE)

The Observatory for Micro and Nanotechnologies aims at PERFORMING A SCIENTIFIC WATCH IN THE DOMAIN OF MICRO AND NANOTECHNOLOGIES. THE WATCH IS BASED ON THE WORK OF MORE THAN 300 EXPERTS, SCIENTIFIC RECOGNIZED RESEARCHERS. THE DOMAIN IS DIVIDED INTO 15 DIFFERENT TOPICS, TWO OF WHICH DEALING WITH “NANOMEDICINE” AND “MICRO & NANO SYSTEMS FOR BIOLOGY”.

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Dendritic glycerol-based amphiphiles: A novel class of solubilizing agents for poorly water-soluble drugs

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Solubilization currently represents one of the major challenges in drug development because approximately 40% of the new compounds are poorly water-soluble. This is of particular concern in the field of parenteral delivery, but also impacts oral delivery and drug development in general. The range of approved excipients used as solubilizers in parenteral formulations is limited to a select few such as Cremophor (R) EL, polysorbate 80, poloxamers, or cyclodextrins.
We designed and evaluated dendritic glycerol-based amphiphiles as novel solubilizers using the poorly water-soluble anticancer drug Sagopilone. The aggregates in water were characterized by DLS, SLS, cryo-TEM and surface-tension measurements. The effect of different core structures on the solubilization, formulation stability, and cytotoxicity using human umbilical vein endothelial cells (HUVECs) were investigated and compared to standard excipients.
Structurally, all amphiphiles were composed of 2nd generation polyglycerol (PG[G2]) as the hydrophilic part and a single C18-chain (PG[G2]-C18), a C18-chain coupled by a diaromatic spacer (PG[G2]-DiAr-C18), a C18-chain with a naphthoyl or bisphenyl end group (PG[G2]-C18-Naph/-BiP), or two C18-chains (PG[G2]-(C18)2) as the hydrophobic part. They formed small (7–10 nm), monodisperse (PDI 0.04–0.20) micelles with the exception of PG[G2]-(C18)2. The amphiphiles revealed a 2–3-fold higher solubilization of Sagopilone than Cremophor® ELP and polysorbate 80 independent of the core structure. PG[G2]-DiAr-C18 exhibited the highest solubilization capacity (56.7±1.3 mg/g) compared to Cremophor® ELP (18.5±0.1 mg/g). The micellar dispersions were stable in drug content over 3 days (≥97%). In contrast to polysorbate 80, dilutions did not show any precipitation after 3 days at 37 ◦C (remaining drug content: >95%). They did not induce significant cytotoxicity at a concentration of 0.01 g/L after 24 h, and PG[G2]-C18-Naph was the least cytotoxic structure after 72 h with values comparable to Cremophor® ELP and polysorbate 80. Overall, these amphiphiles possess superior solubilization properties compared to standard excipients used in parenteral formulations with an excellent formulation stability profile and comparable cytotoxicity

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Inhibition of HUVEC movement by ZnO, SiO2 and Ni nanoparticles
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Episodes of elevated concentrations of particulate air pollution are linked with increase in morbidity and mortality because of respiratory or/and cardiovascular diseases. It has been proved that airborne nanoparticles (<100 nm) can translocate across pulmonary epithelium into the bloodstream, where they either interact with vascular endothelium or are redistributed into other sites of the body. Time-lapse analyses of cell migration were conducted in order to investigate the effect of ZnO, SiO2 and Ni nanoparticles on the motility of HUVEC cells. Representative trajectories of cells moving under control conditions and in the presence of
nanoparticles are illustrated in circular diagrams. Analysis of the individual trajectories showed that both the speed of cell movement and cell displacement were inhibited by all three types of nanoparticles in a dose-dependent manner.

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Numerical modeling and experiments of bio-inspired micro-swimming robots
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Autonomous micro-swimming robots can be utilized to perform specialized procedures such as in vitro or in vivo medical tasks and micro manipulation. Nature provides guidelines for the conceptual design of the micro-swimming robot, which is composed of a body that carries a payload, control and actuation mechanisms, and a long flagellum, which can be either a whip-like tail-actuator that deforms and propagates sinusoidal planar waves similar to spermatozoa, or a rotating rigid helix similar to many bacteria, such as E. Coli.

Navigation and control of the robot are key requirements to ensure successful completion of its task. In micro fluidic environments, dynamic trajectories of active micro-swimming robots must be predicted reliably and the response of control inputs must be well-understood. Reduced-order models, which are based on the resistive force theory (RFT), prove useful to predict the transient, coupled rigid body dynamics and hydrodynamic behavior of bio-inspired artificial micro-swimmers. In the reduced-order model of the micro-swimmer, fluid's resistance to the motion of the body and the tail are computed from RFT, which breaks up the resistance coefficients to local normal and tangential components. Simple theoretical results under simplifying assumptions (such as the absence of fluidic interaction in the flagella and between the body and the flagella) and 3D transient computational fluid dynamics (CFD) simulations can be utilized to obtain RFT coefficients. CFD simulations of a single degree of freedom swimmer are used to predict RFT coefficients of the micro-swimmer with a spherical body and flexible tail actuator, which uses traveling plane wave deformations for propulsion.

Reduced-order models are validated by means of direct observations of natural micro swimmers presented earlier in the literature. Coefficients obtained from CFD simulations perform much better than the theoretical results obtained under simplifying assumptions.