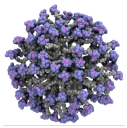

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Cancer Nanomedicine – from the Bench to the Bedside

October 15-17, 2019, Bellevue Park Hotel, Riga, Latvia

BOOK OF ABSTRACTS

Oral Communications

Day 1

Prof. Nissim Garti is a full professor Emeritus of chemistry and the former director of the Casali Center of Applied Chemistry, The Hebrew University of Jerusalem, Israel.

Garti was announced as a 'distinguished professor' and is a '**Ratner Family Chair of Chemistry of the Hebrew University' (since 2011).**

In 2013, Garti was recognized by the Hebrew University as one of the 23 most influential inventors for his contributions in Food Science, Nutraceuticals and Delivery of bioactives. Garti is a member of the 'Hall of Fame' of the University.

Garti was awarded many international and national prestigious prizes. Among the important recent awards are: Life-Time Scientific Achievements in Food Science (AOCS, Orlando, 2015); Surfactants in Solution Society Award (Coimbra, Portugal 2014); Supelco/Nicolas Pelick Award (St Antonio, USA, 2013); Chang Award of the American Oil Chemists Society (Montreal, 2011).

Garti was twice the Director of the Casali Institute, and the Director of the School of Applied Science and Technology. He served (2010–2013) on the Board of Directors of the Hebrew University and is a member of Boards of other academic institutions.

Garti's scientific achievements include over ***400 peer reviewed publications, 13 edited books, 85 chapters in books and over 90 patents.*** 65 PhD and 95 MsC students have graduated under Garti's supervision.

In the last few years Garti has devoted much of his time in a new stratup (Lyotropic Delivery Systems, LDS) that specializes in formulations of nanodomains as delivery vehicles for pharmaceuticals.

Novel Nano Domains as Multi Purpose Delivery Vehicles for Bioactives

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In recent years, a very significant effort is made to design and prepare novel nano delivery vehicles for insoluble, large molecular bioactive and chemically sensitive pharmaceuticals.

The novel nanostructures have to be versatile, based on FDA permitted ingredients, fully dilutable in aqueous medium, thermodynamically stable, and easy to prepare.

The number of generic bioactives with very limited bioavailability and poor performance is very large. Many drugs have shown poor performance. Now it is recognized that the poor performance might be due to poor bioavalability or chemical instability.

Many novel formulations are proposed by R&D centers of pharma companies and by scientists, yet only few are crossing the Phase iii FDA safety and performance requirements.

We will report in this presentation on novel "Molecularly Engineered Modified Liquid Nano Domains" that are demonstrating enhanced bioavalability for a few selected drugs used in four different applications (Oral. Dermal. Transdermal, Ocular and Intravenous).

The results of these studies have led to the establishment of successful Drug Delivery Labs (Named LDS) showing promise in solubilization and membrane crossing of several generic and innovative pharmaceuticals.

Some examples will be discussed including CBD regioselectively extracted from cannabis flowers.

Designing Patient-Specific Nanodrug-Protein Corona

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Enhancing targeting of nanodrugs to destiny cells involves improving its stability in circulation and evading the immune system. Nanodrugs entering the circulation are adsorbed by biomolecules in the body fluid forming a very dynamic entity called protein corona. The nanodrug targeting and uptake is highly dependent on the composition and distribution of proteins on the surface of the protein corona [1]. However, most efforts have been focused on rationale design of nanodrugs, but a rationale design of protein corona not yet have been explored. Our lab has been studying nanoparticles protein corona with proteomics based methods, including the development of SUSTU, a method to characterize the surface of the corona that is direct interactor with target cells [2, 3]. We have studied the evolution of the protein corona by incubating nanoparticle subsequentially in two different media, confirming that the corona maintains features acquired in their initial corona. Here, we hypothesize that including the formation of a patient-specific protein corona previously to nanodrug administration would increase the nanodrug stability and targeting capability. Using rat as animal model and applying shotgun proteomics and SUSTU, we have studied: the evolution of the nanoparticle protein corona and its surface; the application of a depletion method to enrich the serum in low abundant proteins; the incubation of the nanoparticle with enriched serum and characterization of the composition and distribution of the protein in the surface of the corona; the evolution of the corona surface after incubation of the designed corona with the original serum; and the effect of the phenotype variability. Our results suggest that a rationale design of a patient-specific nanodrug-protein corona could offer a novel solution to improve nanodrug targeting and uptake by cells.

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From Drug Discovery to Preclinical Studies: SINTEFs Nanoparticle-Based Technologies for Cancer Treatment

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The Department of Biotechnology and Nanomedicine at SINTEF has extensive experience within design, development and characterization of nanoparticles for drug encapsulation and delivery towards cancer treatment. Within this field of research, we cover the whole value chain from drug discovery to preclinical studies (Figure 1).

We have several novel and patented drug delivery platforms based on degradable polymer materials. The materials are used for nanoencapsulation of hydrophobic drugs and biologics for improved bioaccumulation and controlled release in tumors. The platforms cover passive targeting of solid tumors; active targeting to cancer cells; ultrasound-mediated drug delivery; and long-lasting injectables. Many of these have been extensively characterized and studied both *in vitro* and in preclinical cancer models, and the main results from these studies will be presented here.

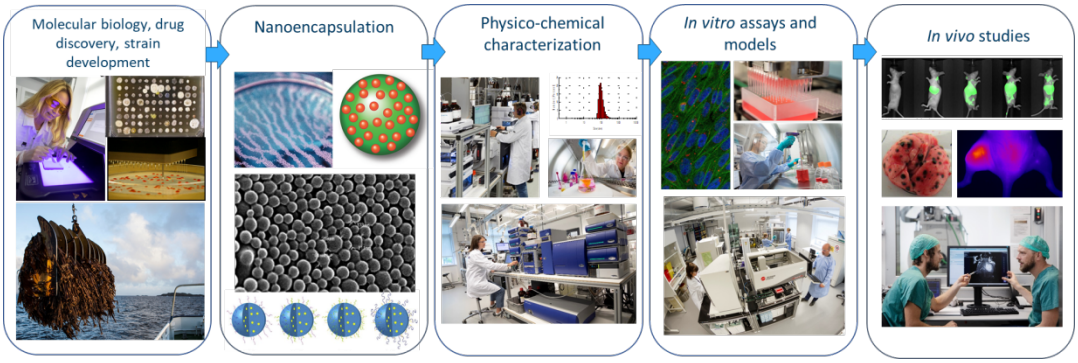

Figure 1. From drug discovery to preclinical studies at SINTEF

The SINTEF group currently both leads and is a partner in a large number of national and international projects on development of nanocarriers for delivery of cytostatic drugs and biologics (proteins, DNA/RNA). SINTEF is a core partner and assay group leader in EUNCL, the European Nanomedicine Characterization Laboratory, having high expertise and state-of-the-art instrumentation for chemical characterization (composition, drug loading, drug release in complex media, contamination, etc) and high-throughput and/or high content cell-based *in vitro* analyses, in 2D and 3D format.

Bio-Polymeric Nanoparticles as Hydrophobic Drug Carriers for Biomedical Application

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Nanoparticles composed of polymers (PNPs) are referred to as nanospheres. The nanospheres have a matrix character, i.e. their entire mass is solid and molecules (e.g. drugs) may be adsorbed at their sphere surface or encapsulated within the polymer matrix. The range of the nanosphere's size is from 10 to 1000 nm. Biodegradable and biocompatible polymer-based nanoparticles (bio-PNPs) are frequently applied in nanomedicine and biotechnology to improve the therapeutic value of various drugs. Drug's nanoencapsulation in bio-PNPs increases their e.g. bioavailability, solubility, retention time, efficacy, tolerability, and drug therapeutic index. However, one of the most important factors is the size of applied nanosystems (100–200 nm), which determine their efficacy in biomedical applications.

In the presented studies, the focus was on developing the most beneficial method of the preparation of hydrophobic drug-loaded bio-polymeric NPs, with the size within 100–200 nm, as the nanosystem for medical application. The PNPs were synthesized using nanoemulsion-template techniques combined with evaporation of an organic solvent method. The polymer-based NPs were made of different types of biopolymers: polycaprolactone (PCL), polylactic acid (PLA), and polylactide-co-glycolide (PLGA). Drug's nanoencapsulation was performed using an example of a hydrophobic model drug (Coumarin-6). Furthermore, selected PNPs were functionalized, using layer by layer approach, by creating polyelectrolytes shell composed of PLL (poly L-lysine) and PGA (poly-glutamic acid), and with an external layer of PEG (poly(ethylene glycol)). The synthesized PNPs were characterized by size distribution (Dynamic Light Scattering (DLS) technique) and zeta potential (Laser Doppler Electrophoresis (LDE) technique) measurements (Zetasizer Nano ZS, Malvern Panalytical Instruments). The concentration of PNPs(aq) suspension was determined by Nanoparticles Tracking Analysis (NTA) using NS500 NanoSight (Malvern Panalytical Instruments). The drugs encapsulation was confirmed by UV-Vis spectroscopy using UV-1800 (Shimadzu) and spectrofluorimetry using FluoroLog-3 (Horiba, Jobin-Yvon). The morphology of synthesized bio-polymeric nanoparticles was determined by cryo-SEM imaging (FE-SEM, Jeol Ltd).

The obtained results allowed to select the most beneficial method of the preparation drug-loaded PNPs that can be further modified/functionalized as nanosystem for biomedical application.

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Barium Ferrite Magnetic Nanoparticles Labeled with ^{223}Ra: a New Potential Magnetic Radiobioconjugate for Targeted Alpha Therapy

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^{223}Ra, as radium chloride, is the first commercially and widely used α-radiopharmaceutical. It is easily obtained from the ^{227}Ac/^{223}Ra generator. However, ^{223}Ra is used only for treatment of bone metastases derived from primary prostate and breast cancers. Unfortunately, lack of an appropriate bifunctional ligand for radium was the reason why ^{223}Ra has not yet found application in receptor targeted therapy. Because Ra^{2+} and Ba^{2+} are nearly identical cations; in our studies we propose to use barium ferrite ($BaFe_{12}O_{19}$) nanoparticles as multifunctional carriers for ^{223}Ra radionuclide for targeted α therapy.

Barium hexaferrite nanoparticles labelled with ^{223}Ra were synthesized by a modified autoclave method described by Drofenik et al. [1]. The reaction mixture of $FeCl_3$, $BaCl_2$ and $^{223}RaCl_2$ was alkalized with NaOH solution and stirred in autoclave at 210^{o}C for 6h. Obtained magnetic $BaFe_{12}O_{19}$ nanoparticles were characterized by transmission emission microscopy and dynamic light scattering. The diameter of synthesized nanoparticles was ~20 nm and the determined magnetization of nanoparticles in room temperature was about 42 emu/g. Yield of labelling was about 70 % (for 100 kBq ^{223}Ra). Stability of the obtained radioactive nanoparticles was tested in various biological solutions: 0.01M PBS, 0.9 % NaCl and in human blood serum. It is confirmed that ^{223}Ra was highly retained inside nanoparticles in every tested solution. Only about 20 % of ^{211}Pb (recoiled decay product of ^{223}Ra) was found in solution.

In order to synthesize a radiobioconjugate having affinity to HER2 receptors, the monoclonal antibody trastuzumab was conjugated to the obtained barium ferrite nanoparticles using phosphonate linker. Synthesized bioconjugate was characterized by thermogravimetric analysis, dynamic light scattering and were tested for stability in biological fluids. The obtained [^{223}Ra]$BaFe_{12}O_{19}$-CEPA-trastuzumab radiobioconjugate almost quantitatively retains ^{223}Ra and majority of the daughter products. In-vitro biological studies indicate that [^{223}Ra]$BaFe_{12}O_{19}$-CEPA-trastuzumab exhibits high affinity and cytotoxicity to the to the SKOV3 ovarian cell line.

We have shown that radium ferrite nanoparticles labelled with ^{223}Ra and functionalized with trastuzumab presents a prospective solution for the use of the ^{223}Ra as a therapeutic tool for targeting HER2 positive breast and ovarian cancers.

Acknowledgement: This work was supported by National Science Centre of Poland by Grants NCN Preludium 2015/17/N/ST4/03943 and Opus 2016/21/B/ST4/02133.

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Liposomal-Based T_2 MRI contrast agents

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Up to now, majority of the iron oxide-based clinically approved contrast agents for magnetic resonance imaging (MRI) have been withdrawn from the market either due to safety concerns or lack of profits. Therefore, there is a need for novel imaging agents with high safety margin and superior MRI properties. Several factors influence relaxation of water molecules in the vicinity of the magnetic centres, such as NPs magnetization and surface coatings. The latter can affect the relaxation of water molecules in various forms, such as diffusion, retention, hydration, and hydrogen bonding. In the first part, it will be demonstrated how size and clustering influence nanoparticles magnetic properties and consequently relaxivity r_2 values. In the second part, the focus will be on the coating optimization with a description of all the parameters that influence r_2 values and thus the performance of NPs as T_2 MRI contrast agents. Proper surface coating endows NPs with good colloidal stability and protects them from undesired degradation or aggregation. Effect of different coating material and thickness on the r_2 values will be discussed. Moreover, a surface that favors diffusion and retention of water molecules within the second sphere is preferred. Taking all these parameters into account, the case study made on different phospholipids as optimal coating material will be presented. In conclusion, the *in vitro* MRI measurements reveal that use of magneto liposomes as contrast agents leads to an improvement in the contrast and an easier distinction between the healthy and the cancerous tissues, proving that the developed liposomes have a high potential to be used as the MRI contrast agent even at very low concentration.

Magnetic Antimicrobial Nanodrugs: Functionalized Cobalt Ferrite/Gold Nanocomposites

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Gold/ferrite nanocomposites are characterized by extensive possibilities to tailor surface properties (including reactivity, affinity, charge, and wettability, to list a few among many others) that could be very effectively optimized to design specific interactions with biological membranes. Their magnetic nature and high stability could be very effectively employed for designing transportability, specific crossing of biological barriers and targeted delivery of drugs.

Cobalt-ferrite/gold nanocomposites investigated in this study contained dihydrocaffeic acid-functionalized cobalt-ferrite and arginine-functionalized nano-gold. Initially hydrophobic, oleic-acid stabilized nanoparticles (NPs) were turned into hydrophilic by exchange of the oleic with dihydrocaffeic acid. Hydrophilic cobalt-ferrite NPs were used as templates for precipitation of nano-gold with the surface functionalized by cationic arginine amino acid. Interactions with bacteria were tested in *S. epidermidis* as Gram positive and *E. coli* as Gram negative bacterial strain. More stable NPs were capable of more intensive interactions with bacterial membranes. Nanocomposites affected bacterial growth depend on: presence of cationic arginine molecules on the surface of nano-gold as well as content of dihydrocaffeic acid on the top of cobalt-ferrite NPs. According to Live/Dead staining and SEM analysis, pore formation and disintegration of the bacterial wall were confirmed as a consequence of synergic effect of arginine and dihydrocaffeic acid on the surface of nanocomposite interacting with the surface of bacteria. Interactions of the nanocomposites and their compatibility with mammalian cells were tested in red blood cells (RBCs) isolated from sheep blood. A very low level of hemolysis has been obtained for nanocomposites with different compositions of capping molecules. RBCs exposed to concentrations of NPs that induced most intensive antimicrobial activity were morphologically compact. There was a difference in interactions of functionalized cobalt-ferrite/gold with bacterial in comparison to their interactions with mammalian cells. Last confirmed a possibility to design selectivity of these NPs depend on the nature and composition of interacting membrane.

A possibility to design interactions of cobalt-ferrite/gold nanoparticles with biological membranes is giving these multifunctional nanoparticles an exceptional possibility to design nanodrugs and to tailor their therapeutic efficacy.

G-quadruplexes Nanosystems for Cancer Therapy

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Cancer disease is one of the major burdens for health systems of developing countries. The current treatments are surgery, radiotherapy and chemotherapy. These methods are invasive, with limited effectiveness and undesirable side-effects, and regrettably, a high percentage of the patients still die.

G-quadruplexes, besides being a potential drug target, have also been used as potential cancer therapeutics. AS1411 (Antisoma, London, UK) is a guanosine-rich 26-base G-quadruplex-forming oligonucleotide aptamer that can induce apoptosis and thus inhibit the growth of malignant cells and reached under Phase II clinical trials for the cure of renal cancer and acute myeloid leukemia. This aptamer has been shown to have cancer-selective properties against a variety of malignant tumors, ensuring the effective delivery of other drugs or imaging agents used in combination therapies. Thus far, AS1411 has been exploited as an active targeting ligand in the construction of a variety of nanosystems (nanoparticles, carbon dots, dendrimers, liposomes, micelles). However, despite good tolerance and safety profile, it is highly polymorphic, folded into multiple, essentially mono- but also bimolecular G-quadruplex structures and the trial was terminated due to suboptimal pharmacokinetics (rapid clearance) and low potency.

Herein, we studied AS1411 aptamer derivatives [1, 2, 3] that adopted a single G-quadruplex structure in solution and RNA G-quadruplexes [4] for the delivery of ligands with anticancer potential into cancer cells. The ligands used were acridine orange (AO) derivatives (C_3, C_5 and C_8) that showed potent inhibitory effect towards cancer cells, and when combined via supramolecular with the G-quadruplexes lowered the ligand's cytotoxicity towards non-malignant cells. The conjugates G-quadruplexes-ligands were prepared and characterized by circular dichroism, nucleic magnetic resonance, fluorescence spectroscopies.

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New CD44v6-Targeted Treatment for Advanced Colorectal Cancer Mediated by Niclosamide-Loaded Polymeric Micelles

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Colorectal cancer (CRC) relapse and therapy-resistant metastases formation are sustained by the presence of cancer stem cells (CSC) within the tumor [1]. The specific delivery of chemotherapy to tumors by targeted drug nano-delivery systems (nanoDDS) that could also target this sub-population represents a promising approach to improve treatment efficacy. Recently, CD44v6 demonstrated to be a robust biomarker for advanced CRC and CSC, due to its functional role in tumorigenesis and cancer initiation process [2]. We propose CD44v6-functionalized polymeric micelles (PM) loaded with niclosamide (NCS), a drug with activity against CSC [3], as a strategy to face CRC metastatic disease. HCT116 cells were sorted accordingly the CD44v6 receptor expression into CD44v6+ (high expression) and CDv44v6– (low expression). As expected, CD44v6+ cells presented stemness properties, such as the capacity to form bigger colonspheres. The designed PM loaded with NCS and functionalized with a CD44v6-Fab presented adequate features in term of size, charge, and encapsulation efficiency. Moreover, functionalization specifically increased their internalization by the CD44v6+ cells. Encapsulation of NCS improved drug effectiveness *in vitro*, and permitted the administration of 8 times more drug *in vivo*. Most importantly, in *in vivo* biodistribution studies, functionalized PM accumulate in tumor for at least 48h. The designed PM have the potential to serve as a platform for the development of new therapies for CRC treatment, particularly in the prevention of CSC-driven cancer recurrence. The easy and scalable manufacturing as well as the safe and biodegradable nature of the selected polymers are crucial factors that would allow an easy translation and implementation of these nanoDDS into the clinical practice.

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Messenger RNA-Mediated Anticancer Drug Release in Mesenchymal Cells by Spherical Nucleic Acids

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Nanoparticulate systems are of great interest for applications in biomedicine due to ability to design their properties. The ligand coating of the nanoparticles is critical for nanoparticle stability and function while the morphology and chemical composition of the nanoparticle core is important in defining optoelectronic, magnetic and other properties of the particles. In recent years, advances in nanoparticle chemical synthesis and surface functionalization rendered available a library of functional nanomaterials. The current step of evolution is the synthesis of nanomaterials that perform multitasking roles triggered by external stimuli. Such designs will be of high importance in biomedicine, especially for targeted and efficient drug delivery.

This presentation will discuss recent progress in our group concerning the design of nanoparticle assemblies and their incorporation in biological systems to facilitate sensing, drug delivery and accurate manipulation. The talk will focus on a new class of nanoparticle dimers that can accommodate multiplexed synergistic actions of sensing and drug delivery in cells [1–7]. These multitasking particle assemblies are able to selectively release anticancer drugs in response to specific messenger RNA signatures and selectively kill model cancerous cells as opposed to healthy cells.

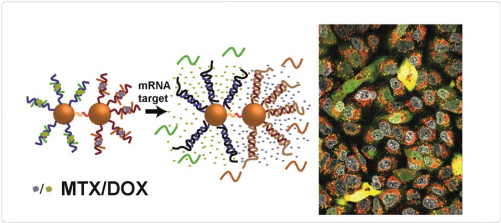

Figure. DNA-gold nanoparticle dimers for multiplex sensing and drug release in cells.

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Inhibition of Selected P450 Cytochromes by Carbon Nanostructures

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For the last decade, different carbon nanostructures have been tested for various applications in nanomedicine, including cancer therapies. They have been studied as a potential treatment, as well as a platform for bioactive molecules. In some cases, despite their effectivity, lack of direct cytotoxicity or even lack of systemic toxicity, possible interactions with molecules within tissues after long exposure to nanostructures should be considered. In our studies, we focused on three allotropic forms of carbon – diamond nanoparticles, graphite nanoparticles and graphene and its derivatives, mostly graphene oxide. During previous experiments, it was proved that these nanostructures did not cause significant toxic effects in rats after long-term exposition and they were deposited within tissues and probably transported to the liver [1]. Liver is the main organ responsible for drugs and other xenobiotics transformation, so possible interactions with liver proteins needed to be studied further, with emphasis on cytochrome P450 enzymes. Using microsome-based model, we showed that diamond and graphite nanoparticles, as well as graphene oxide platelets, inhibited activity of CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4 enzymes, expressed by decreased formation of the final products of enzymatic reactions. Moreover, *in vitro* studies on HepG2 and HepaRG cell lines showed that the same nanostructures also down-regulated gene expression of some of the enzymes on mRNA level in cells [2]. Also, graphene oxide had the most significant impact on the enzymes among the tested nanostructures. The obtained results suggest that application of the carbon nanostructures for internal *in vivo* applications requires a special attention to possible interactions with cytochrome P450 enzymes. Because of their unique structure they might interfere with the mentioned enzymatic reactions, leading to alterations in the level of products formed by P450 cytochrome, including changes in metabolism of co-administrated drugs.

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Assessment of Genotoxic and Pro-Apoptotic Effect of Various Nanoparticles on Human Endothelial Cells

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Development of technology enables production of nano-scale structures in submicroscopic sizes, with specific properties, such as small size, large surface area to mass ratio or high reactivity. These structures are used in many areas such as medicine (as potential transporters of drugs in targeted therapy), engineering (production and application of nanocomposites) or cosmetology (mineral UV filters in cosmetics). With the increasing application of nanomaterials there is also a risk associated with the presence of nanoparticles in the environment which residues. Since nanoparticles easily enter into the body, they can be distributed into various organs and tissues. However, the endothelial cells are the ones which create the first barrier penetrated by the various nanostructures.

Due to increasingly widespread application of nanoparticles in the modern world, there is an urgent need for further specialised nanotoxicological studies. The main aim of this study was the assessment of cytotoxicity of nanoparticles (with various charge or diameter), which belong to three different groups: polymers (EDTMP, MDP), monoclonal antibodies (Trastuzumab) and nanoemullsion (modified and nonmodified with chitosan). Our experiments were carried out on human immortalized vein endothelial cells, HUVEC-ST cell line. To assess a cytotoxicity of investigated nanoparticles (by Neutral Red and Alamar Blue assays), we compared a cell viability of HUVEC-ST cells after the treatment with the examined nanoparticles. We assumed that nanemulssion possess the most cytotoxic effect towards normal endothelium cells. Their high cytotoxic index was referred to the significant number of apoptotic and necrotic cells as well as the morphology's alterations which appeared in the cell culture after treatment of HUVEC-ST cells with the examined nanoparticles. Following changes of the cellular homeostasis were related to the decrease of mitochondrial membrane potential (MMP) and an activation of caspase-3. Genotoxic properties of nanoparticles were assessed by employing comet assay, TUNEL assay and the phosphorylation of H2AX histone. The results of our experiments confirmed that tested nanoparticles induced DNA damage (the most significant observed in probes treated with nanoemullsion) which caused DNA double strand brakes and its olionucleosomal fragmentation.

Innovative Nanoplataform with UCNPs for Multimodal Therapy of Melanoma

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Upconverting nanoparticles (UCNPs) have emerged as promising systems for cancer diagnosis and therapy, due to their unique NIR-driven imaging/drug delivery/therapeutic applications, including high tissue penetration, minimal photodamage, high photostability, absence of photoblinking or photobleaching, weak autofluorescence and high signal-to-noise ratio [1, 2]. As melanoma is the most aggressive form of skin cancer and one of the most challenging malignancies to treat with limited and non-curative options in conventional clinical treatments, the importance of new therapeutic approaches for multimodal therapy of melanoma becomes undeniable [3].

Therefore, we aim to produce a totally innovative nanoplataform formed by NIR excitable UCNPs with triple therapeutic approach for melanoma therapy: hyperthermia; reactive oxygen species; and antitumor drug delivery, respectively in a context of photothermal therapy (PTT), photodynamic therapy (PDT) and chemotherapy.

For that purpose, SrF2:Yb/Er UCNPs and NaYF4:Yb/Er UCNPs were synthesized and coated with a mesoporous silica shell to allow the incorporation of the tumor targeting molecule, the photosensitizer, and the antitumor drug. Characterization results regarding size (TEM, DLS), surface charge (zeta potential), composition (EDS and FTIR) and preliminary results of functionalization with folic acid (FA) are presented. Viability assays was used to test this nanoplataforms in melanoma cell lines.

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Synthesis of Biologic-Responsive Polymers for Manufacturing of Docetaxel-Loaded Nanoparticles for Glioblastoma Chemotherapy

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Glioblastoma (GBM), among brain cancers, is with the highest prevalence and mortality worldwide. Docetaxel is one of the most effective chemotherapeutics against GBM, although it presents pharmacokinetic constraints mainly due to its low solubility and poor blood-brain barrier (BBB) permeation. This project proposes a biologic-responsive nanomedicine solution to circumvent these inadequacies through nanoparticles (NPs) loaded with docetaxel for glioblastoma treatment. The developed nanomedicine comprises a poly(lactic-co-glycolic)acid (PLGA) core and a polyethylene glycol (PEG) shielding of long and short length. The long-length PEG possesses Angiopep-2 moiety for BBB targeting (low-density lipoprotein receptor) and is able to dissociate in the acidic pH of BBB endosomes, hence sterically deprotecting the short-length PEG coupled with L-histidine for further GBM targeting (L-type amino acid transporter 1) upon brain arrival. The chemical synthesis achieved a total purity value of around 70 % and 90 % for the long- and short-length PEG, respectively, as demonstrated by different characterization techniques such as nuclear magnetic resonance (NMR), Fourier-transform infrared spectroscopy (FTIR), gel permeation chromatography (GPC) and matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF). Docetaxel-loaded NPs were produced post-polymer synthesis through a microfluidic technique using 80 %, 10 % and 10 % of PLGA, long- and short-length PEG, respectively. The physicochemical characterization of the NPs demonstrated an average size of around 100 nm, polydispersity index of 0.1, zeta-potential of -15 mV and entrapment efficiency of 60 %.

Overall, the current need to accelerate drug delivery to glioblastoma, bypassing the BBB and targeting tumour tissue of brain, places this system in a privileged position in the field of translational nanomedicines. This work also lays foundation for future biologic-responsive delivery of other therapeutics to a range of pathologies.

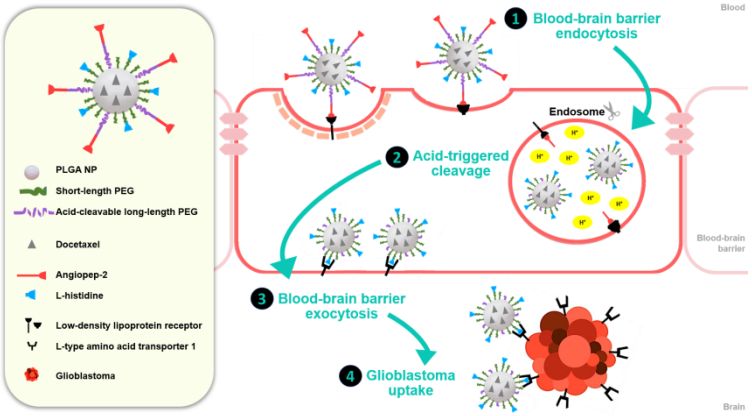

Figure. Graphical abstract

Porous Silicon-Based Nanomedicine: Fantasy or Future

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Cancer diagnostic and therapy challenge the scientific community to design research addressing the urgency of ending cancer. The intention of the reserach is to study development and in vitro testing of biocompatible and biodegradable porous silicon-based nanostructures for cancer, bacteria and viruses medical treatment, as shown in Figure 1. These novel nanostructures are based on insights gained during the last years of research in the study group and are expected to lead to significant progress steps, by which such material will be promoted from "promising material" to effective material for the biophotonics and biomedical applications. For all these reasons new labelling and drug delivery agents for bio-application are an important field of research with a growing potential for medical usage. Si-based nanomaterials (silicon nanowires (SiNWs), porous silicon nanoparticles (pSiNPs)) are a type of novel bionanomaterials with attractive properties including excellent optoelectronic and mechanical properties, favourable biocompatibility, huge surface-to-volume ratios, surface tailorability, improved multi-functionality, as well as their compatibility with conventional Si technology. The multi-modal bioimaging of silicon nanoparticles in human cancer cell models will be presented and discussed in details [1–4]. The novel treatment concepts in cancer, bacteria and viruses therapy using ultrasound and radio-frequency irradiation will be discussed in details.

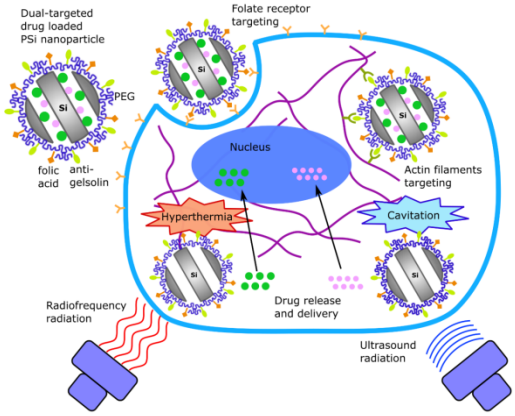

Figure 1. Schematic representation of drug delivery concept based on dual therapeutic effects using dual targeted/chemotherapeutic loaded biocompatible porous silicon nanostructures, activated by therapeutic ultrasound or radiofrequency radiation.

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Graphene Nanoplatform as Selective Gene Delivery System in Glioblastoma Cancer Research

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Glioblastoma (GBM) is the most common, primary and aggressive tumour of brain cancer. Patients' survival time is extremely low, and it does not exceed five years [1]. Despite improvements in chemotherapy, radiation, surgical techniques, novel therapies are in need to improve the clinical investigation of GBM tumours and extend survival of patients. Gene delivery therapy mostly use the viral vector, which has seriously advert events in gene therapy, including immunogenicity, inherent toxicity, tumorigenicity, or induced malignancy from retrovirally based gene therapies [2]. However, disadvantages of viral vector can be efficiently replaced by nanocarriers. Nanocarriers can reduce or even eliminate the side effect of viral carries, with high efficiently of microRNA or antisense RNA delivery to GBM cells and tumour tissue.

The objective of this study was the usage of graphene based nanoplatforms to induce deregulation of mircoRNA level in glioblastoma cancer cells and tumour tissue and to activate the p53, PTEN, PUMA protein and inhibit the Pi3K/AKT signalling pathway. The nanoplatforms were characterized by FTIR, scanning transmission electron microscopy and Zeta potential. The efficiency of microRNA delivery to the cancer cells were analysed by the flow cytometry. The effect of anticancer activity of graphene based nanoplatform functionalized by microRNA sequence were analysed by MTT assays and at the gene expression level. The obtained results partly explain the mechanisms of microRNA deregulation stress, affected by, by graphene based nanoplatforms together with forced transport of miR-7, miR-124, miR-137 and antisense miR-21, -221 and -222 as an anticancer supportive therapy.

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Mechano-Signalling, Induced by Fullerene C_{60} Nanofilms, Arrests the Cell Cycle in the G2/M Phase and Decreases Proliferation of Liver Cancer Cells

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Degradation of the extracellular matrix (ECM) changes physicochemical properties and dysregulates ECM-cell interactions, leading to several pathological conditions, such as invasive cancer [1]. Carbon nanofilm, as a biocompatible, easy to functionalise and nano-sized material, could be used to mimic ECM structures, changing cancer cell behaviour to perform like normal cells [2].

Experiments were performed *in vitro* with HS-5 cells (as a control) and HepG2 and C3A cancer cells. An aqueous solution of fullerene C_{60} was used to form a nanofilm. The morphological properties of cells cultivated on C_{60} nanofilms were evaluated with light, confocal, electron and atomic force microscopy. Both cell viability and proliferation were assessed by XTT and BrdU assays. Immunoblotting and flow cytometry were used to evaluate the expression level of proliferating cell nuclear antigen and determine the number (percentage) of cells in the G2/M phase.

All cell lines were spread on C_{60} nanofilms, showing a high affinity to the nanofilm surface. It was found that C_{60} nanofilms mimicked the niche of cells, were biocompatible and non-toxic, but the mechanical signal from C_{60} nanofilm created environment that affected the cell cycle, which lead to reduced proliferation.

In studies on liver cancer cells, it was documented that the signal derived from the fullerene nanofilm is preferentially chosen by the cell, creating environment conducive to adhesion and colonisation. Furthermore, cells settled in this way decreased the ability to form spheroids, caused the cell cycle arrest in the G2/M phase and decreased proliferation. It can be expected that the incorporation of fullerenes in the ECM of liver cancer cells can reduce cell malignancy and improve tumour therapy.

This work was supported by grants NCN2016/Z1/3/N29/01029 and NCN2016/23/D/NZ7/03837

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Carbosilane Glycodendrimers: From Synthesis to Bioapplications

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Dendrimers (DDMs) as a class of symmetric nanoparticles are studied for their promising applications in biomedicine [1, 2]. Cationic DDMs, mostly investigated in gene delivery due to their ability to form complexes with negatively charged nucleic acids, also exhibit relatively high toxicity [3]. Glycodendrimers (glyco-DDMs) with carbohydrate peripheral moieties proved to be a suitable alternative to positively charged DDMs. Still, the toxicity issues are widely discussed.

Here we present new synthetic pathways towards carbosilane glycodendrimers decorated with glucose and galactose derivatives with a high potential for bioapplications. In addition, to our best knowledge, we performed the first *in vivo* toxicological data (modified FET, Zebrafish embryos) for novel 1st–3rd generation glucose glyco-DDMs (DDM_1Glu, DDM_2Glu, DDM_3Glu) and their comparison with the traditional *in vitro* cytotoxicity assays (MTT, 3 types of rodent cell lines). Overall, the modified FET revealed two to three orders of magnitude difference between the *in vivo* and *in vitro* toxicity of the tested glyco-DDMs [4].

While, in general, the glyco-DDMs are of great promise as efficient vectors in drug/gene delivery, their developmental toxicity should be further investigated.

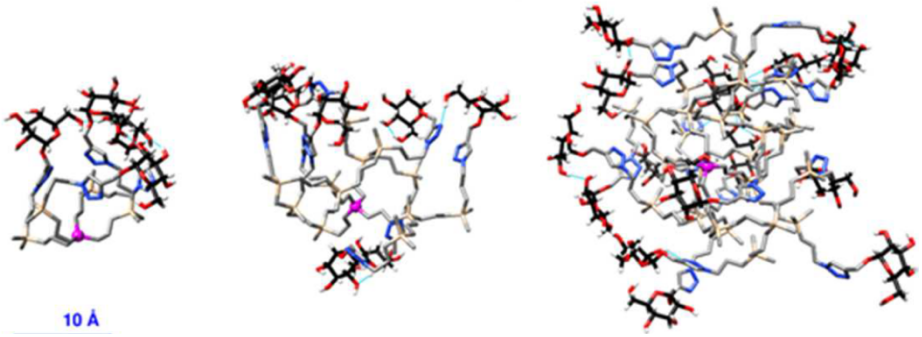

Figure. Computer models of $DDM_{1\text{-}3}Glu$

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Oral Communications

Day 2

Moein Moghimi is a Professor of Pharmaceutics and Nanomedicine (School of Pharmacy) and Research Professor (Institute of Cellular Medicine) at Newcastle University (UK); Adjoint Professor at the Department of Pharmaceutical Sciences, Skaggs School of Pharmacy and Pharmaceutical Sciences, University of Colorado-Denver Medical Center (USA); and Deputy Editor of Molecular Therapy (Cell Press). Professor Moghimi is a co-founder of S. M. Discovery Group LLC (Colorado, USA) and S. M. Discovery Group Ltd. (UK). His earlier appointments have included Professor and Chair in Pharmaceutics at the School of Medicine, Pharmacy and Health, Durham University, UK (2016–2017); Full Affiliate Member/Professor at the Methodist Research Institute, Houston Methodist Hospital Systems, Houston, Texas, USA (2013–2017); Visiting Professor at Università Degli Studi Di Padova, Padova, Italy (2015); Professor of Nanomedicine (at the Department of Pharmacy), Professor of Pharmaceutical Nanotechology (at the NanoScience Center), and Founder/Director of the multi-million Dollar Center for Pharmaceutical Nanotechnology and Nanotoxiocology, University of Copenhagen, Denmark (2008–2016); and Honorary Professor of Nanomedicine at the Multidisciplinary Research Center, Shantou University, China (2008–2010).

Prof. Moghimi's research is centred on fundamental and translational aspects of nanomedicine engineering and performance, with the overall goal of advancing fundamental understanding of biological barriers, and particularly the role of innate immune system, in relation to nanoparticle performance and safety, and within the context of precision medicine applicable to cancer, cardiovascular diseases, immune disorders, and disease of the central nervous system. As of 2019, Prof. Moghimi has over 270 peer-reviewed publications/patents in the field and has given over 400 invited talks, keynotes and plenaries.

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Selected recent representative publications:

Nature Nanotechnology **14:** 629–635 | *Nature Nanotechnology* **14:** 260–268 | *Molecular Therapy* **26:** 933–934 | *ACS Nano* **11:** 11584–11593 | *Nature Nanotechnology* **12:** 589–594 | *Nature Nanotechnology* **12:** 387–393 | *ACS Nano* **11:** 12–18 | *Biomaterials* **112:** 141–152.

Nanomaterials and Innate Immunity: the Complement Challenge

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The complement system is a central constituent of the innate immunity, not only acting as a functional bridge between innate and adaptive arms of the immune system, but also linking immune system with the coagulation system. Nanomaterials often trigger complement activation in blood through complex and multifaceted mechanisms [1–3]. This has ramifications in nanomaterial stability, pharmacokinetics and therapeutic performance [4]. This presentation will discuss the role of complement in nanoparticle opsonisation and macrophage clearance as well as in disease progression, paying particular attention to disparities in immune handling of nanomaterials among different species. Finally, equivocal investigations have indicted a role for the complement system in infusion reactions to nanomedicines (termed CARPA: *Complement-Activation Related PseudoAllergy*), where the extent of nanomedicine-mediated complement activation in human serum is biasedly and curiously correlated to cardiopulmonary responses in pigs. Contrary to these, a large body of evidence suggest that the porcine reactions are related to robust nanoparticle clearance by pulmonary intravascular macrophages (PIMs) and rapid release of arachidonate metabolites from these cells regardless of complement activation [4–6]. The "CARPA spin", therefore, is the best representative of *Complement-Assumed Related Performance Anomaly*. Similar to pigs, other animals that have resident PIMs in their lungs also respond to intravenously injected particles, where rapid particle clearance by PIMs correlate with peak periods of cardiopulmonary distress. Notwithstanding, global nanomedicine safety assessment in the porcine model (and other ruminants and species with PIMs) is inappropriate and misleading, and these models should not be advertently promoted and their applications exaggerated. These issues will also be discussed.

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Development of Apoferritin Formulations for Antitumour Drug Delivery

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Despite promising activity, poor aqueous solubility and bioavailability limit therapeutic application of pre-clinical antitumour agents; for clinical anticancer drugs, adverse systemic toxicity is often dose-limiting. The apoferritin (AFt) protein cage has been proposed as a stable, biocompatible, biodegradable drug delivery vehicle[1], facilitating passive targeting and accumulation within the tumour microenvironment due to enhanced permeation and retention (EPR). Moreover, up-regulated transferrin receptors (TfR1), a feature of many cancer cells, mediates endocytosis of AFt and its encapsulated cargo.

We use AFt to encapsulate a number of anticancer agents to achieve their targeted delivery, enhanced uptake and sustained release. The encapsulation of tyrosine kinase inhibitor gefitinib leads to intracellular drug accumulation, dose-dependent growth arrest and inhibition of colony formation in SKBR3 breast cancer cells[2]. The experimental antitumour agent 2-(3,4-dimethoxyphenyl)-5-fluorobenzothiazole (GW 610) and its amino acid ester prodrug (GW 608-Lys) exert potent, selective antitumour activity *in vitro*[3]; however, GW 610 is lipophilic and poorly soluble. AFt-encapsulated GW 610 (190 molecules per cage) demonstrated enhanced antitumour activity ≥3-fold in sensitive colorectal carcinoma cell lines[4]. Encapsulation of GW 608-Lys enabled markedly increased encapsulation (>380 GW 608-Lys per AFt) and release <24 h. AFt/GW 608-Lys was sequestered more rapidly than naked agent by cancer cells and >90-fold more potent anticancer activity was achieved.

A barrier to successful treatment of intractable brain tumours (glioblastoma multiforme, GBM) is the blood brain barrier, consisting of endothelial cells that express TfR1. We demonstrate that encapsulation of the standard of care chemotherapeutic alkylating agent temozolomide (TMZ; ~500 molecules per AFt) enhances its *in vitro* antitumour activity in isogenic U373V (vector control) and U373M (MGMT+; resistant to naked TMZ) GBM cell lines, demonstrating that AFt-TMZ overcomes TMZ-resistance.

AFt-formulations represent a promising biocompatible drug delivery system capable of targeted drug delivery and sustained drug release. Additional therapeutic and targeting modalities can be introduced by modification of the protein capsule[5]. The obtained results indicate realistic prospects for exploitation of these new formulations and merit further *in vivo* pre-clinical evaluation.

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Synthesis of Peptidic Bola-Dendrimers

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Dendrimers are potential non-viral vectors for efficient delivery of drugs and nucleic acids to cancer cells. However, biomedical applications of such macromolecules require high quality synthesis and well determined structures [1]. Recently, dimeric bola-type molecules, where two bulky fragments are connected with flexible spacer, have aroused a great deal of attention since it was discovered that such unique structures improve complex stability and transfection efficiency [2, 3].

It is known that synthesis and purification of dendrimers is still a challenge. Herein, we report a developed methodology for synthesis of amphiphilic cationic dendrons based on poly-lysine or poly-ornithine scaffolds, functionalized with various organic residues to achieve in one molecule high affinity for cell membranes and the ability to form supramolecular complexes with drugs or siRNA molecules. Obtained dendrons were combined with chemically stable or biodegradable linkers to create dimeric amphiphilic "bola" structures. Efficacy of synthetic methods in solution or on solid support and coupling reactions (active ester method vs. the Schotten-Baumann method) were compared.

Furthermore, after purification and characterization by NMR, mass spectroscopy (MS) and analytical HPLC, HPLC complexation experiments of bola-dimers with known anticancer drugs were performed.

During the presentation, the selected examples of methodology for synthesis of dendrimers will be briefly presented and discussed.

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Effect of Particle Shape on Interactions of Gold Nanoparticles with Proteins of Different Glycosylation Status

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Systematic and comprehensive study of the nano-bio interface is necessary to provide further insight into specific aspects of biological applications of nanoparticles (NP), which will subsequently help in future design of NPs for nanomedicine [1–3]. When NPs are introduced to a biological medium, numerous interactions occur at the nano-bio interface. Upon contact with biological matrices, NPs become immediately coated by a layer of biomolecules, in particular a large panel of proteins that form a protein corona (PC). The structure and composition of the PC depends on the physicochemical properties of the NPs, the nature of the physiological environment, and the duration of exposure [4, 5]. Despite extensive investigation of interaction between NPs and proteins, the role of protein glycosylation on formation, characteristics and fate of PC-NP complexes is almost completely overlooked.

This study aimed to examine the effect of protein glycosylation on the mechanism of PC formation on the surface of gold NPs designed as theranostic systems. The model system consisted of gold NPs of different shape and with different surface chemistry, and glycosylated human transferrin and non-glycosylated recombinant transferrin as model proteins. Fluorescence spectroscopy was employed to monitor intrinsic fluorescence quenching of proteins due to their adsorption on the NP surface. The binding constants were calculated from titration experiments using the Stern-Volmer equation.

The obtained results clearly demonstrated that mechanism of PC-NP formation is dependent on both physico-chemical characteristics of NPs and protein glycosylation status.

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Graphene Oxide Nanoplatelets Decrease Glioma Induced Angiogenesis

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Gliomas, which are some of the most common malignant tumors of the central nervous system, develop a microenvironment that is characterized by an altered redox state and an abundance of proangiogenic and proinflammatory factors [1]. Gliomas develop an expanded vessels network and angiogenesis pathologies including vascular hyperproliferation and hemorrhage caused by the breakdown of the intratumoral blood–brain barrier [2]. Proangiogenic signals in tumors are fueled by cycling hypoxia, reactive oxygen species, reactive nitrogen species, acidosis, and inflammation [3]. Tumor cells, including gliomas, maintain an altered redox environment with high production of ROS and RNS that causes tumorigenic cell signaling [4]. Reactive oxygen species and reactive nitrogen species can influence tumor cell malignancy via the redox-regulated transcription factor NF-κB, whose activation is further regulated by the mutation status of p53 [5].

The objective of this study was to assess the influence of graphene oxide nanoplatelets on the angiogenic potential of glioma cell lines with different p53 statuses. Nanoparticle treatment decreased the angiogenic potential of U87 (p53 wild type) but not U118 (p53 mutant) cells. Nanoparticle activity was related to the decreased level of intracellular ROS and RNS, which downregulated NF-κB signaling depending on the p53 status of the cell line. Activation of NF-κB signaling affected downstream protein levels of interleukin 6, interleukin 8, growth-regulated oncogene α, and monocyte chemotactic protein 1.

These results indicate that the activity of graphene oxide nanoplatelets depend on mutation status of glioma cells and therefore give new insights into the use of nanoparticles in personalized biomedical applications regarding glioma angiogenesis and its microenvironment.

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Liposomes – Nanotechnological Plataform for Different Clinical Applications

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Since the first description of vesiculated phospholipid systems in 1965 by Alec Bangham, more than 50 years of research allowed to transform those vesicles from membrane models into successful drug delivery systems [1, 2]. Many nanotechnological advances are based on this evolution and nowadays liposomes are being used in diverse areas to deliver antibiotics, anti-cancer, anti-inflammatory and anti-parasitic drugs, anaesthetics, macromolecules, such as enzymes and even for theranostic applications. This success is due to unique properties of liposomes that are biodegradable and biocompatible, their ability to be manufactured with different mean sizes and properties, enabling the incorporation of different kind of molecules irrespectively of molecular weight, electric charge or solubility, possibility to interact with cells, protect the entrapped drugs from inactivation, optimize the *in vivo* biodistribution profile of the associated drug and consequently to improve its therapeutic efficacy.

Some examples of liposomal formulations will be presented either using already commercial drugs, such as rifabutin, paromomycin and doxorubicin, where an improvement of their *in vivo* antimycobacterial [3], antiparasitic [4] and anticancer [5] effect was achieved, or new synthesized molecules, with cytotoxic properties towards tumor cells, which in pre-clinical studies showed remarkable antitumor activity in melanoma models particularly for nanoformulated compounds [6, 7].

Versatility for producing liposomes according to the therapeutic purpose and/or route of administration will be presented. The development of novel liposomal formulations acting as delivery systems and/or protectors of the incorporated material amplifying their activities and reducing their degradation, constitutes a stimulating research area. These strategies can raise interest of pharmaceutical companies allowing the introduction of novel liposomal products in the market.

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Gateway to Brain: Tailored Nanomedicine

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Research of non-invasive therapy for treatment of neurodegenerative diseases is one of the most important topics of the last years by the pharmaceutical technology [1–3]. Even if less than 1 % of both industrial and university research projects on neuroscience displays of a Blood-Brain Barrier (BBB) crossing and CNS targeting aims, the study and progress of drug delivery strategies to cross the BBB are supposed to be widely addressed.

Above a wide overview on the most interesting and recent applications of nanomedicines to the CNS targeting, in this talk, the most recent works on poly-lactide-co-glycolide and other polymer-based NPs differently modified for BBB crossing will be reviewed. In particular, different strategies based on different ligands for BBB crossing, as exogenous-like peptides, endogenous-like peptides BBB-receptor antibodies and glyco-peptides will be detailed [4, 5]. *In vivo* and *in vitro* results will be commented to underline which mechanism is responsible for BBB crossing, which pathways are exploited for cell entry and specific accumulation-tropism in brain areas and even in cell type are present, dependently on type of ligands.

With this talk, we will therefore try to draw an overview of the main advantages of the use of nanomedicine-based approach for innovation in crossing the most “defensive” barrier in our body, with particular relevance to neurodegenerative diseases. Besides these aspects, a critical analysis on the main causes that slow the application of nanomedicine to brain disorders will be discussed along with the identification of possible solutions and possible interventions.

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Functional Hybrid Material Using Electrospun Polymeric Framework and Functional Elements for Cancer Therapy

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Hybrid materials have emerged as a solution for bringing infrequent properties within a framework of 1-dimention (1D) and 2-dimension (2D) structures. For instance, Hybrid electrospun nanofibers are a powerful tool to tailor 2D framework which has the potential to include incompatible drug entities within polymeric matrix. These provide the opportunity for supporting multiple functional domains such as nanoparticles within biological macromolecules. These functional entities could be simply synthesized using laser ablation and wet chemical method synthesized *in-situ*, within the electrospinning solution which offer additional interaction sites within the nanofibers.

Nanofibers have been characterized using microscope, thermogravimetric analysis, spectroscopy and among other specific analysis techniques to reveal the potential impact in wide range of theranostic applications. Polymers like chitosan, poly (ethylene oxide), polycaprolactone have been utilized which are generally bio-compatible and biodegradable hence, are much suited for biomedical applications. Consequently, nanofibers range from 100 nm at the lower end of diameter spectrum to just short of micrometer depending on the parameters chosen for electrospinning and physio-chemical properties of polymer and functional elements.

This green approach of producing nanofibers provides that potential to be used in regenerative medicine as it is environment friendly. In addition to the fibers' profile providing higher surface are to volume ratio, another incentive to use nanofibers comes from the controlled, tunable and targeted drug release capacity [1, 2]. The hybrid nanofibers electrospun also using co-axial strategies have been shown to possess high surface area and much higher controlled reactivity beneficial for many applications including surface tissue engineering and theranostic of cancerous cells by inducing hyperthermia using various of nanoparticles.

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Development and Applications of Nanodispersions as Carriers of Compounds with Pharmaceutical Interest

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Delivery of insoluble or slightly soluble chemotherapeutic agents remains a major issue in cancer therapy. In this context, non-ionic, oil-in-water (o/w) microemulsions were developed to encapsulate and deliver lipophilic compounds with antitumor activity, namely PLX 4720 and DPS-2 [1]. PLX 4720 is a commercially available analogue of Vemurafenib, an anticancer drug for melanoma that targets mutated $BRAF^{V600E}$ kinase. DPS-2 is a pharmaceutical benzothiophene analogue initially designed to inhibit $BRAF^{V600E}$ and thus, exhibited significant cytotoxic activity towards various cell lines tested. About 50 % of melanoma cases, present activated BRAF mutations and the majority of them refers to V600E [1]. In this context, the developed microemulsions are proposed for dermal delivery.

The delivery system was structurally characterized employing Dynamic Light Scattering (DLS), Cryogenic-transmission electron microscopy (Cryo-TEM) and Electron Paramagnetic Resonance (EPR) spectroscopy. The results revealed that the size of oil droplets was not affected upon encapsulation. Both compounds were found to be located within the oily cores of microemulsions without interacting with the surfactant monolayer [2]. Additionally, effective release of the drug from the oil phase was evaluated by Confocal Microscopy. The cytotoxic effect of both microemulsions and their cargo was examined through the MTT cell proliferation assay. The microemulsion, as carrier, had no effect on cell line viability while PLX4720 and DPS-2 exhibited cytotoxicity in all cell line when loaded in microemulsions. The mechanism of the DPS-2-induced cell death was investigated through Fluorescence-activated cell sorting (FACS) analysis, Comet assay and Western Blotting of specific cell death and apoptosis markers. Overall, DPS-2 was effectively encapsulated and delivered within cells inducing a non-apoptotic cell death. DPS-2 is not a direct genotoxic agent, nevertheless, S-phase delay was observed in all cell lines tested implying that DPS-2 is a DNA replication inhibitor [3].

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Advanced Theranostic Systems Based on Nanostructure Materials

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Nanostructured delivery and diagnostic systems that induce specific targeting properties by exploiting local physicochemical tumor characteristics will be evaluated is the present work [1, 2]. It is well known that cancer cells have specific physicochemical characteristics, which can be taken into consideration for the design of a broad spectrum of drug delivery systems (DDS). Some of those characteristics include the different temperature environment, its susceptibility when temperature ranges between 40–43 °C where cell apoptosis is induced, the intra- and extra- cellular pH which varies from 6.0 to 6.8, for cancer cells, and 6.5 to 7.4 for normal cells respectively, (lysosomes acidic pH ranges 4–5). Additional significant factors are the overexpressed receptors on the tumor surface [2, 4].

Loading and release studies were carried out by using the anthracycline drug Doxorubicin and their cytotoxicity was evaluated by using the MTT assay in healthy and diseased cell lines.

The highlight of this work is the *in vitro* and *in vivo* study which was performed in order to evaluate different nanostructures as for their biodistribution, pharmacokinetic and toxicity per se.

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Creating Self-Illuminating Nanoconjugated Nanoparticles (Nanosystems) to Treat Pancreatic Cancer

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Pancreatic cancer has a very high mortality rate, due to the low response to current treatments [1]. Therefore, the development of new strategies to address this tumor are essential. Photodynamic Therapy (PDT) has proven to be efficient for treating external lesions such as melanoma. Besides, this therapy provides a new approach to its treatment and has some proven advantages compared to chemotherapy, radiotherapy and inmunotherapy [2]. However, due to the poor penetration of light, PDT is not suitable for treating internal organs, like pancreas. For this particular purpose, we aim to determine the potential of self-illuminating nanoparticles [3], to mediate a localized and controlled antitumoral response in pancreatic cancer.

Self-illuminating nanoparticles were developed by the conjugation of a mutant version of Renilla reniformis luciferase (RLuc8) to quantum dots (QDots). The functionality of RLuc8 was determined by measuring its bioluminescence intensity after adding its substrate (coelenterazine), showing robust activity. The conjugation of the protein to the quantum dots (RLuc8-QDots) was performed by EDC coupling, forming an amide group between the primary amine group of the protein and the carboxylic groups set on the surface of the QDots [4]. Conjugates were characterized by gel electrophoresis and measuring its bioluminiscence spectral scanning for calculation of BRET [5]. The stability of this nanosystem was also tested through the time, measuring its BRET kinetics. The interaction of the conjugates with pancreatic cancer cells was analyzed by confocal microscopy.

The conjugation of RLuc8 to QDots was successfully achieved, as demonstrated by agarose electrophoresis; while free QDots freely migrate through the gel, the conjugates were stuck at the loading wells. Second analysis by polyacrylamide gel electrophoresis confirmed the absence of free unreacted RLuc8. Additionally, functionality of the conjugates was evidenced by determining their BRET ratio after the addition of the substrate (BRET ratio = 5.05). The nanosystem was stable up to seven days. The formation of Reactive Oxygen Species (ROS) after addition of coelenterazine and RLuc8-QDots, to activate the photosensitive drug verteporfin, is currently under study.

Thus, we have characterized self-illuminating nanoparticles that are efficiently internalized into pancreatic cancer cells. Because of this efficient internalization and considering the optimal BRET properties of the conjugates, we expect that they will efficiently activate a photosensitizer, generating ROS and driving cancer cells into apoptosis.

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Different Rational Nanotherapeutic Approaches Towards Superficial Cancers

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Different approaches aiming a targeted and more efficient treatment of superficial cancers like melanoma have been developed by our research group [1]. One approach involves functionalized gold core nanoparticles (NPs) which have shown great flexibility for obtaining multifunctional systems due to their plasmonic tunable properties, thermal activation by laser in the near-infrared zone (650–900 nm) and specific surface functionalization [1, 2]. The other approach involves polymeric core NPs as carriers for a new anti-cancer drug: PvD [3]. In the present study, the preparation method of both NPs, full physico-chemical characterization, efficiency and *in vitro*/*in vivo* safety assessments of two multifunctional NPs were performed. Both NPs were successfully developed and showed small size and spherical morphology. In case of Gold NPs, this treatment allowed a tumor volume reduction of around 80 % in xenograft model, after one single treatment. In some animals, this treatment led to formation of several necrotic foci, observed after histological analysis. No significant skin erythema at the irradiation zone was verified, nor for other excised organs. The *in vivo* preliminary studies conducted with the PvD-loaded polymeric core NPs showed that the tumors had an extensive area of necrosis and were also highly hemorrhagic (> 90 %) after two-weeks of treatment. Internal organs, such as, heart, lungs, liver, spleen and kidneys did not show any structural change, suggesting a very localized tumor cell inoculation. The study of molecular mechanisms of both NPs on melanoma is still ongoing.

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Polysaccharide-Graft-Poly(2-Alkyl-2-Oxazoline) Hybrid Copolymers for Nanomedicine Applications

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We prepared a family of modular "molecular toolbox-like" hybrid copolymers polysaccharide-*graft*-poly(2-alkyl-2-oxazoline) by a simple one-pot procedure consisting of cationic ring-opening polymerization of 2-alkyl-2-oxazoline monomers terminated with sodium alkoxide of the corresponding polysaccharide. The copolymers may then be conjugated to functional moieties via the terminal double bonds. The poly(2-alkyl-2-oxazoline) parts were hydrophilic (to fine-tune biodegradation and cell uptake rate) or thermoresponsive (enabling the formation of local depots upon heating to body temperature), respectively. The polysaccharides were glycogen (fully biodegradable D-glucose-based hyperbranched structure), β-glucan and lentinan (D-glucose-based Toll-like receptor agonist immunomodulators), κ-carageenan (anionic thermo- and potassium-responsive immunomodulator) or mannan (DC-SIGN receptor targeting ligand to macrophages and dendritic cells), respectively. We demonstrated tuneable multistimuli-responsive behavior of aqueous solutions of these hybrid polymers. These polymers were also demonstrated to show superior anticancer effects as local radioimmunomodulators in *in vivo* murine model [1], as sentinel lymph node [2] or melanoma imaging agents in in vivo murine models, respectively.

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Polymeric Nanoparticles for Theranostic

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The term "theranostic" is defined as a material that combines modalities of therapy and diagnostics. Thus, theranostics deliver therapeutic drugs and diagnostic imaging agents at the same time within one dose. That approach has the potential to overcome undesirable differences in biodistribution and selectivity that currently exist between distinct imaging and therapeutic agents. More than 40 % of pharmacologically active compounds exhibit poor solubility due to their hydrophobic character that results in poor bioavailability. Nanoencapsulation is one of the most established approaches to increase bioavailability of poorly water-soluble drugs and their delivery to the place of the action. Drug containing nanocarriers can be further functionalized for theranostic.

The aim of this work was to develop a method of preparation of polyelectrolyte nanocarriers for the theranostic application. The method of formation nanocarriers containing selected hydrophobic drug e.g. paclitaxel was developed, moreover such nanocarriers were further functionalized for theranostic by incorporation MR imaging agents e.g. iron oxide nanoparticles or gadolinium complex. Nanocarriers were prepared by sequential adsorption technique (layer by layer). Formation of the polyelectrolyte multilayer was evidenced by zeta potential changes with a number of layers. Nanocarriers were optimized/modified for passive drug delivery systems. The PEG corona on the top polyelectrolyte layer assured the stability of nanocapsules against aggregation in the media with high ionic strength (FBS solution) was formed. The size of the formed theranostic nanocarriers was ~150 nm. The empty theranostic nanocarriers did not show any deleterious effects on tested cell lines (CT26-CEA, B16F10, 4T1 and PBMC), whereas encapsulated drug Paclitaxel retained its cytotoxic/cytostatic activity. Using T2 and T1 NMR relaxation measurements with 9.4T preclinical MRI scanner, we demonstrated that our nanocarriers can be detected due to a locally altered contrast in the MR image. Thus, they may become a promising platform for future theranostic applications.

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Liposomes for Micro-RNA Delivery

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Cancer and atherosclerosis are chronic inflammatory diseases considered as main causes of deaths worldwide. One risk factor involved in the progression of disease is obesity. A novel strategy to combat obesity is the recruitment of thermogenic adipocytes. Only recently, a micro-RNA (miR-26a) was identified by our group [1] as a regulator for white and brite/beige adipocyte differentiation. Micro-RNAs are the most studied class of non-coding RNA molecules that play a central role as regulators of gene expression. However, to be applied as novel therapeutics micro-RNAs need proper delivery systems to achieve efficient intracellular uptake.

To face this challenge we have synthesized and characterized diverse cationic liposomal formulations for the encapsulation of miR-26a. Parameter like particle size, surface charge and entrapment efficiency were determined by dynamic light scattering, nanoparticle tracking analysis, zetapotential and native PAGE electrophoresis. The silencing efficiency of the formulations was evaluated in comparison to commercially available transfection reagents in human multipotent adipose-derived stem (hMADs) cells. A key marker and readout for the silencing efficacy of the micro-RNA loaded liposomal delivery system was the enhancement of uncoupling protein 1 (UCP1). Liposomes composed of cationic lipids (DOTAP), fusogenic helper lipids (DOPE) and neutral lipids (POPC) optionally stabilized by PEGylated lipids were most efficient. The liposomes showed particle sizes in the range of 100–150 nm and a zetapotential of about 40–45 mV. The highest incorporation efficiency was achieved for highly positively charged liposome formulations and an optimal lipid to micro-RNA ratio of 360:1 mol/mol could be determined. The addition of polycations like polyethylenimine or protamine had no effect. Best transfection results in cell culture were achieved at lipid concentrations between 1.8–3.6 mg/ml corresponding to 14–28 µM lipids on cells.

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Radiolabeled Magnetic Nanoparticles for Cancer Theranostics

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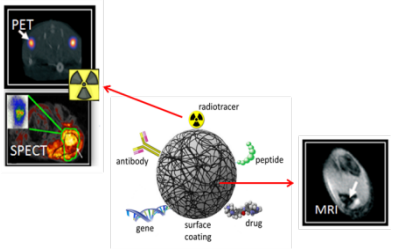
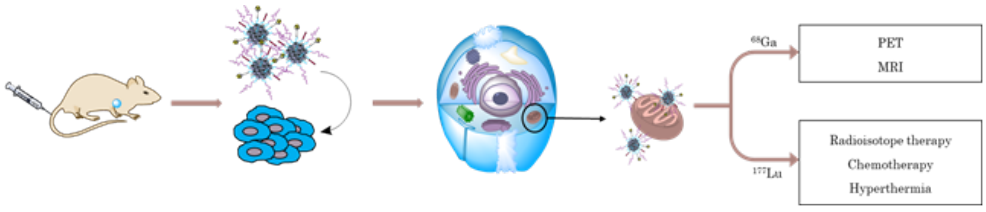
Cancer theranostics is a relatively new term that encompasses both diagnosis and treatment. There are plenty of hindrances of conventional diagnostic and therapeutic agents, compromising not only the therapeutic process but even the life of the patient. Therefore, a new approach for the development of more evolved, cancer-specific and effective theranostic tools is proposed in this presentation.

Dual-modality contrast agents, such as radiolabeled nanoparticles, are promising candidates for a number of diagnostic applications, since they combine advantages of two different imaging modalities, namely SPECT or PET imaging with MR imaging. The benefit of such a combination is to more accurately interpret disease and abnormalities *in vivo*, by exploiting advantages of each imaging technique.



A novel anticancer theranostic consisting of magnetic nanocrystals coated by a polymeric shell of alginic acid in which PEG chains are attached, is evaluated *in vitro* and *in vivo*. The polymeric shell allows further functionalization with DOX, as well as the THP chelator for labeling with the radioisotopes Gallium-68 and Lutetium-177. A mitochondria-targeting agent is also conjugated to the nanoparticles, which will lead to selective accumulation of nanoparticles to cancer cell mitochondria. The proposed nanoplatform will be capable of dual-modality imaging with PET and MRI. Furthermore, a triple therapeutic effect can be achieved, after targeted delivery of the functionalized MNPs, attributed to the simultaneous presence of the chemotherapeutic agent, the therapeutic radioisotope and the application of magnetic hyperthermia.

In the current presentation, we provide an overview of our recent work concerning synthesis and characterization of the nanoplatform, direct radiolabeling experiments with ^{68}Ga as well as some initial *in vitro* and *in vivo* results.



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Silica-Based Multifunctional Nanomedicine for Targeted Drug Delivery

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Mesoporous silica nanocarriers (MSNs) have received a growing attention as drug delivery systems. Due to their stable and rigid framework under variable conditions compared to organic drug delivery systems (DDSs), silica carriers do not suffer from uncontrollable drug release owing to an accelerated degradation in a physiological environment, but have the potential to release the drugs in a sustained way when suitably functionalised [1]. In addition to this, if we vary the synthesis conditions and the type of reagents used, we can modify the structure, composition, and size of pores [2–3]. It is then possible to influence the loading capacity and the release kinetics of the drug we want to load and deliver.

MCM-41 type mesoporous silica nanocarriers were synthesized in a basic environment, using a cationic surfactant as template and tetraethoxysilane (TEOS) as silica precursor to build the network via formation of siloxane bonds. Other silica precursors (organo-substituted trialkoxysilanes) can be added together with TEOS in different ratios during the synthesis, in order to incorporate organofunctional groups to the inorganic silica matrix, directing the particles morphology, pore properties and structure. This is called co-condensation approach (one-pot synthesis). The mesoporous silica phase was finally achieved by removing the template by extraction with hydrochloric acid 1M and ethanol. Every batch of MSNs synthesized was characterized by different techniques. The drug of interest was then loaded into the particles. *In vitro* release studies were carried out in PBS at pH 7.4 and 5.4 in order to test if the mesoporous silica nanoparticles possessed controlled release properties of the payload.

Scanning Electron Microscopy of MSNs synthesized using only TEOS as silica precursor showed particles with a spherical shape and with uniform size distribution around 100 nm. A surface area of 1044 m^2/g and an average pore diameter of 3.3 nm was calculated. Moreover, the typical hexagonal mesopore order of MCM-41 nanoparticles was confirmed by powder X-ray diffraction. The synthesized particles showed typical MCM-41 features and a release of the loaded drug.

We will keep selecting and optimizing the silica precursors, templates and reaction conditions in order to maximize drug loading and to analyze different release profiles that can be obtained with the different functionalization of the silica matrix.

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Targeting Prostate Cancer via PSMA-Peptide Decorated Exosome-Mimetics

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Prostate cancer (PC) is the most common type of cancer and the second cause of death in men worldwide. A range of effective anti-cancer drugs have been used to treat advanced PC; however, their systemic toxicity has limited their clinical use. Therefore, there is an unmet need to develop novel strategies to deliver cancer therapeutics to PC tissues. Exosomes are nano-sized, cell-derived vesicles that carry proteins and RNAs for intercellular communication. They could also deliver their cargo across the plasma membrane and delay premature drug transformation and elimination. Exosomes have shown an intrinsic homing ability to a wide range of cells. Furthermore, a new approach has been proposed to combine the intrinsic homing ability of exosomes with active targeting to enhance their tumour accumulation. In the present work, we report the development of novel prostate specific membrane antigen (PSMA)-targeted exosome-mimetics (EMs) for advanced PC.

Stably transfected PSMA-peptide expressing monocytes U937 cell line was established. PSMA-targeted EMs were prepared by serial extrusion of the transfected U937 monocytes. The PSMA-targeted EMs were characterized by dynamic light scattering (DLS), nanoparticle tracking analysis (NTA), Transmission electron microscopy (TEM), bicinchoninic acid (BCA) assay and western blotting. Furthermore, the binding of the PSMA-targeted EMs to the recombinant human PSMA protein was confirmed by ELISA. Also, the uptake studies were performed using flow cytometry and confocal laser scanning microscopy. Their drug loading capability was assessed by loading doxorubicin and its derivatives. Next, in vivo biodistribution and safety studies of targeted EMs were carried out in C4-2B and PC3- tumour-bearing mice.

The engineered EMs exhibited high protein yield, good drug loading and exosome markers expression. The expression of PSMA targeting peptide and its binding to PSMA receptors was confirmed *in vitro*. Finally, successful tumour accumulation of PSMA-targeted EMs was achieved *in vivo* with the absence of *in vivo* toxicity.

The engineered PSMA-targeted EMs, could offer a promising drug delivery system for PC, based on its drug loading capacity, tumour targeting and safety *in vivo*.

pH-Sensitive Triazine-Carbosilane Dendrimerosomes for Anti-Cancer Drug Delivery

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Supramolecular constructions are promising vehicles for anti-cancer drug delivery due to flexibility of their architecture, high drug loading capacity etc. Among others, amphiphilic dendritic molecules can be used as precursors for therapeutic supramolecular assemblies.

Recently, we have proposed a new class of functional dendritic species – amphiphilic triazine-carbosilane dendrons self-assembling into dendrimerosomes in water medium. The triazine residue in the dendrons' structure acts both as a branching point in the hydrophobic part and a pH-sensitive element. Upon protonation, it drives morphological rearrangements of supramolecular assemblies (Figure).

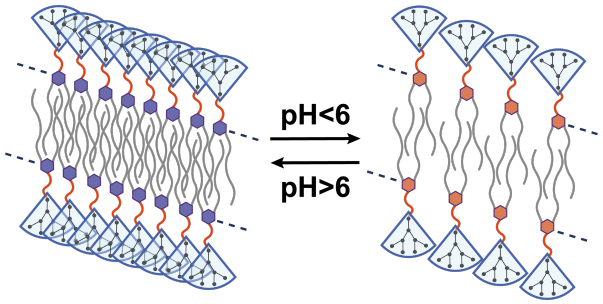

Figure. pH-dependent behavior of triazine-carbosilane dendritic vesicles

Dendrons have been shown to be quite biocompatible at therapeutically relevant concentrations: the IC_{50} is ~8 μM towards healthy donors' PBMCs and ~6 μM towards K562 cells). Dendrimerosomes efficiently encapsulate low-molecular chemodrugs and bind anti-cancer nucleic acids. Currently, we study biological effects of drug-loaded dendrimerosomes in detail. Our preliminary data suggest that novel hybrid dendrimerosomes hold potential for cancer nanomedicine.

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Loading Doxorubicin-PSA Prodrug into Thermo-Sensitive Nanocarriers Enhances its Therapeutic Efficacy in Castration-Resistant Prostate Cancer Models

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Early stages of prostate cancer (PC) have been successfully treated with radiotherapy and/or androgen deprivation therapy. However, overtime androgen-independent cancer cells develop resulting in poor prognosis. Prostate specific antigen (PSA) is clinically the best serological marker for PC, and recently has been exploited as a therapeutic target in advanced PC patients. Doxorubicin-PSA (Dox-PSA) prodrug, L-377202 (developed by Merck), is currently in clinical trials. Intravenous administration of Dox-PSA prodrug could reduce systemic exposure to the active drug; however, it suffers from low water solubility and unfavourable pharmacokinetics, which results in a limited tumour accumulation in metastatic PC lesions. Mild hyperthermia (HT) improves drug bioavailability at the tumour sites, and it has been shown to synergise with several anticancer agents. In this study, we aim to combine Dox-PSA selectivity with temperature-sensitive liposomes (TSL) and mild HT to improve the delivery and the therapeutic efficacy of Dox-PSA prodrug in castration-resistant PC models *in vitro* and *in vivo*.

Dox-PSA prodrugs were synthesized, and loaded into TSL. PSA expression was evaluated in series of androgen-dependent and independent PC cell lines, using Western blot and RT-qPCR. The effect of mild HT effect on PSA expression and cell viability was assessed using Western blot, and resazurin cell viability assay, respectively. Cellular uptake studies in 2D and 3D PC models was evaluated using confocal microscopy and flow cytometry, Finally, the cytotoxicity of liposomal Dox-PSA prodrug in combination with mild HT was determined in 2D and 3D PC models. Therapy experiment was conducted in C4-2B xenografts in combination with mild HT.

Interestingly, HT exposure did not affect cell viability or PSA expression. As expected, Dox-PSA prodrug showed selective toxicity to PSA-expressing PC cells. Moreover promisingly, significant toxicity in both 2D and 3D models was observed following encapsulating Dox-PSA into TSL in combination with mild HT, compared to free Dox-PSA. A significant tumour growth delay was observed with our liposomal formulation in combination with mild HT.

The obtained results show that mild HT could be a promising approach to enhance therapeutic efficacy of liposomal-Dox-PSA formulations in castration-resistant PC models.

Lipid-Coated Upconverting Nanoparticles/Ru(II)-Anticancer Compounds for NIR-Light-Mediated Photodynamic Therapy

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Photodynamic therapy (PDT) being a technique aimed at activating with light a photo-responsive drug is considered a promising alternative to chemotherapy as it might limit side effects and increase treatment efficacy. The biological application of ruthenium anticancer prodrugs for PDT is restricted by the need to use poorly penetrating high-energy photons for their activation, i.e. typically blue or green light. Upconverting nanoparticles (UCNPs), which produce high energy light under near-infrared (NIR) excitation [1] may solve this issue, provided that the coupling between the UCNP surface and the ruthenium prodrug is optimized to produce stable nanoconjugates with efficient energy transfer from the UCNP to the ruthenium complex.

Herein, we report on synthesis and photochemistry of a UCNPs and a Ru(II) polypyridyl complex which is an efficient and photostable PDT photosensitizer. A water-dispersible, negatively charged supramolecular nanoconjugate UCNP@lipid/Ru was prepared by encapsulation of UCNPs in a mixture of amphiphilic phospholipids and complex (Figure 1). A non-radiative energy transfer efficiency of 12 % between Tm^{3+} ions in the UCNP and the Ru^{2+} acceptor was found using time-resolved emission spectroscopy. Under irradiation with NIR light (969 nm), UCNP@lipid/Ru was found to produce reactive oxygen species (ROS) [2].

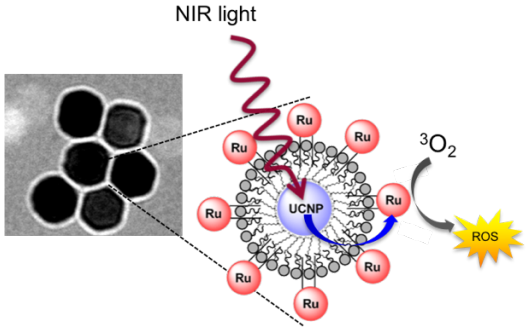

Figure 1. Schematic impression of the nanoconjugate system UCNP@lipid/Ru

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Biocompatible and Versatile Sphingolipid Nanosystems for Personalized Medicine

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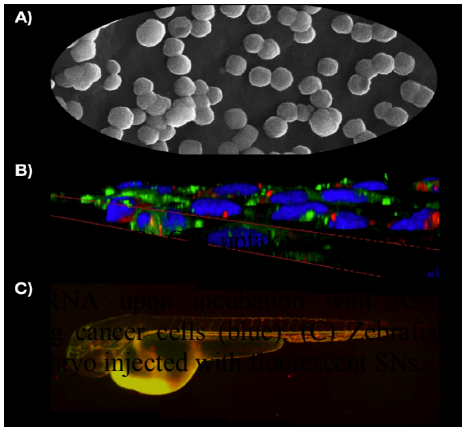
In line with the personalized medicine concept, mainly applied in cancer tretament and also in many other scenarios, there is a growing interest towards the development of versatile nanosystems that can deliver more than one type of drug depending on every patient's needs [1–2]. From a translational perspective, we believe it is very important to develop nanosystems that are easy to prepare and could be adapted to suit industrial requirements, simple in composition, stable, biodegradable, biocompatible, and highly versatile so that they can be useful for the association of different drugs and molecules increasing their therapeutic potential [3].

Sphingolipid nanosystems (SNs) were produced by adapting the ethanol injection method, widely described for the preparation of liposomes. SNs with a mean size around 100 nm, forming a monodisperse population, and with a slightly negative surface charge were obtained (Figure 1A), demonstrated to be highly stable over time and upon incubation with biologically relevant media.

Importantly, *in vitro* experiments demonstrated that they are non-toxic and can efficiently interact with cells to deliver the therapeutic cargo (Figure 1B). *In vivo* experiments, such as those carried out in zebrafish embryos (Figure 1C), proved that SNs are highly compatible upon injection. Importantly, SNs are promising systems in therapeutics, leading to efficient association of different types of drugs and biomolecules (from small hydrophobic drugs to RNAs, aptamers, peptides and proteins), and can also be efficiently radiolabelled for PET and MRI imaging, therefore being of application for the management of cancer and other disease conditions. Finally, experiments were carried out proving that SNs can be decorated with peptides and antibodies for targeting purposes to specific cell subpopulations.



Altogether, we believe we have developed a technology that is highly versatile and can hold a great potential in the era of personalized medicine for the management of cancer and other prevalent diseases.

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Nanomedicine Approach to Glioblastoma Therapy

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Glioblastomas (GBMs) are the most common type of primary brain tumor causing about 4 % of death cases associated to cancer [1]. Glioblastoma cells are genetically unstable leading to a highly infiltrative, angiogenic and resistant to chemotherapy neoplasm. This, along with the fact that chemotherapy is not effective in the long term, leads to poor prognosis with a median survival of only about 14 months from diagnosis, and a 2-year-survival rate as low as 3–5 % [2]. This poor prognosis even when GBM patients are treated according to standard care makes it essential to search for novel therapeutic approaches.

Interference RNA (RNAi) technology is a very effective gene silencing mechanism that is also a very promising therapeutic tool since it can knockdown proteins involved in the pathogenesis of different diseases by targeting their mRNA [3]. One example is cancer where RNAi-mediated knockdown of proteins involved in cancer cell survival has been proposed to potentiate antitumoral actions of drugs [4], establishing another potential therapeutic approach for cancer treatment.

Nanoparticles are able to deliver siRNA to the cell as assessed by using FAM-labelled siRNA and live fluorescence microscopy. Moreover, nanoparticle-mediated siRNA transfection in these cell lines causes about 70 % to 90 % reduction in target proteins in human T98G, mouse GL261 or rat C6 glioblastoma cell lines. This potentiates temozolamide-induced cell death in the cell lines as indicated by an increase in LDH release. Biodistribution studies indicate that the nanoparticle can deliver siRNA to the brain bypassing the blood-brain-barrier.

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Exosome-Mediated RNAi of PAK4 Prolongs Survival of Pancreatic Cancer Mouse Model After Loco-regional Treatment

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Pancreatic cancer (PC) remains one of the most aggressive and devastating malignancies, predominantly due to the absence of a valid biomarker for diagnosis and limited therapeutic options for advanced disease [1]. Exosomes (Exo) as cell-derived vesicles, are widely used as natural nanocarriers for drug delivery [2]. P21-activated kinase 4 (PAK4) is oncogenic when overexpressed, promoting cell survival, migration and anchorage-independent growth. In this study, we validate PAK4 as a therapeutic target in an *in vivo* PC tumour mouse model using Exo nanocarriers following intra-tumoural administration. PC derived Exo were firstly isolated by ultracentrifugation on sucrose cushion and characterised for their surface marker expression, size, number, purity and shape. siRNA was encapsulated into Exo *via* electroporation and dual uptake of Exo and siRNA was investigated by flow cytometry and confocal microscopy. *In vitro* siPAK4 silencing in PC cells was assessed by western blotting, flow cytometry, and *in vitro* scratch assay. *In vivo* efficacy (tumour growth delay and mouse survival) of siPAK4 was evaluated in PC bearing NSG mouse model. Ex vivo tumours were examined using Haematoxylin and eosin (H&E) staining and immunohistochemistry. High quality PC derived PANC-1 Exo were obtained. siRNA was incorporated in Exo with 16.5% loading efficiency. Exo and siRNA co-localisation in cells was confirmed by confocal microscopy. PAK4 knock-down was successful at 30 nM Exo-siPAK4 at 24 h post incubation *in vitro*. Intra-tumoural administration of Exo-siPAK4 (1 μg siPAK4 and 7.7 × 1011 Exo, each dose, two doses) reduced PC tumour growth and enhanced mice survival ($p < 0.001$), with minimal toxicity observed compared to polyethylenimine (PEI) used as a commercial transfection reagent (Figure 1E). H&E and IHC staining of tumours showed significant tissue apoptosis in siPAK4 treated groups. PAK4 interference prolongs survival of PC bearing mice suggesting its candidacy as a new therapeutic target in PC. PANC-1 Exo demonstrated comparable efficacy but safer profile than PEI as *in vivo* RNAi transfection reagent.

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Oral Communications

Day 3

Charm of Dendrimer Nanotechnology for Cancer Therapy

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The application of nanotechnology to engineer nanovectors for drug delivery is widely expected to bring breakthrough and create entirely novel therapeutics for cancer treatment. Dendrimers are ideal nanocarriers for drug delivery by virtue of their uniquely well-defined structure and multivalent cooperativity [1]. We have recently established bio-inspired structurally flexible dendrimers [2] and self-assembled supramolecular dendrimers [3] for drug delivery. In particular, self-assembling dendrimers are able to form modular, adaptive and responsive nanosystems, and effectively deliver various chemo- and bio-therapeutics as well as imaging agents for diagnosis and personalized treatment [3], offering new perspectives in nanotechnology based biomedical applications for personalized medicine.

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Oncolytic Viruses and Extracellular Vesicles for cancer drug delivery

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Cancer is still a leading cause of death worldwide. Despite improvements achieved in cancer therapy, the outcome is still partially ineffective against different cancer types. Therefore, there is a dramatic need to develop novel treatment modalities. Among the novel therapeutic strategies, the use of oncolytic viruses (OVs), which are specifically engineered to selectively infect, replicate in and kill cancer cells, is one of the most encouraging approach. Even though promising efficacy has been observed in *in vitro* and *in vivo* preclinical studies, OVs are often administered intra-tumorally (i.t.), thus many solid tumors cannot be treated using this approach.

Extracellular vesicles (EVs) are nanometer- to micron-sized lipid bilayered vesicles able to shuttle biological molecules, such as OVs, even over longer distances [1]. We demonstrated that loading EVs with OV sand chemotherapeutic agents such as paclitaxel, increased their anti-neoplastic activity compared to the virus or EVs alone thus protecting the OV from immune disruption through their encapsulation into EVs (EV-Virus) [2, 3].

Interestingly, by using *in vivo* and *ex vivo* imaging technologies, as a detection system for the characterization of the whole-body biodistribution of the EV-formulations, we provided evidence that a selective homing of cancer-derived EVs to the neoplastic tissue originating the vesicles exist. Our results show that EVs generated from a tumor type have a tropism for cancers originating from different tissues, and even from different species. This heterologous and cross-species homing capabilities suggests for EVs a wider role in cell-to cell communication during tumor progression, which was not expected before. Our results open new avenues for the selective delivery of diagnostic/therapeutic agents to solid tumours.

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Preclinical Validation of New Hybrid Nanocomposites for Theranostic Applications

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Last year, 9.6 million people worldwide died of cancer, 18.1 million new cases were detected and it is estimated that these numbers will grow annually. Recently, magnetic hybrid nanocomposites (mNCs) have opened new perspectives in biomedical and environmental applications [1] and have emerged as an ideal platform aimed at overcoming multiple barriers found in cancer treatment. A range of different hybrid systems have been proposed within the scientific community as bioactive encapsulating agents and carriers due to their biocompatibility, low toxicity and ability to influence the delivery profile of pharmacological agents [2, 3]. These mNCs are being designed to synergistically combine the drug encapsulation/release capability of the organic matrix with the intrinsic physico-chemical properties coming from the inorganic component.

Here, we present Doxorubicin-loaded magnetic hybrid nanocomposites (mNCs-DOX) synthesize by a simple, versatile and sustainable melt-emulsification method [4, 5] and fully characterized showing excellent heating properties in magnetic hyperthermia (MH), good features as T2-contrast agents in magnetic resonance imaging (MRI) and fantastic performance as magnetically responsive drug delivery vehicles. Finally, these nanocomposites have also been pre-clinically validated through *in vitro* and *in vivo* studies in melanoma tumours.

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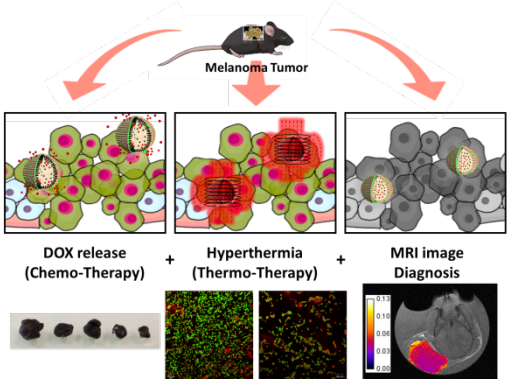

Figure 1. *In vitro* an *in vivo* validation of these mNCs-DOX

Nanoparticles for Cancer Nanomedicine

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Nanotechnologies and nanoscience are new types of disciplines that transform classical disciplines, for example, chemistry, biology, physics, and molecular modelling into new approaches for creating tools and drugs for innovative nanomedicine. In this regard, it is very important to study the possible application of nanoparticles as gene carriers for anticancer small interfering RNA (siRNA) as a useful tool to treat cancer. We studied different nanoparticles such as phosphorus AE dendrimers, ruthenium metallodendrimers, PEGylated gold and silver nanoparticles, "nanoflowers" as gene carriers for nucleic acids. Their bioavailability and interaction with proteins and nucleic acids were evaluated. The internalisation and effect on cancer cell lines of anticancer siRNA transfected by studied nanoparticles was analyzed. The data showed that AE2G3 and AE2G4 dendrimers were effective in transfection of siRNA into cancer cells and non-cytotoxic. Metallodendrimers differed in their action on cancer and normal cells. The application of other nanoparticles – gold and silver nanoparticles, and "nanoflowers" as gene carriers for anticancer nucleic acids need further studies.

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Aptamer-Functionalized Iron Oxide and Gold Nanoparticles for Breast Cancer Theranostics

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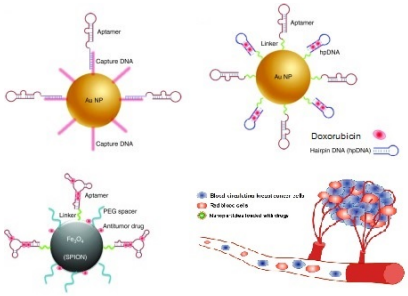
Breast cancer is the second leading cause of death in women worldwide. The extremely fast development of metastasis and ability to develop resistance mechanism to all the conventional drugs make them very difficult to treat which are the causes of high morbidity and mortality of breast cancer patients. Development of superparamagnetic gold-coated iron oxide nanoparticles (Au-SPIONs) functionalized with an aptamer was implemented to allow target delivery of the anticancer drug of doxorubicin (DOX) on triple-negative breast cancer cells. Iron oxide nanoparticles (SPIONs) and gold nanoparticles (GNPs) were prepared by ultrasound-assisted and controlled seeded growth synthetic methods, respectively. The aptamers AS1411, ERaptD4 and H2 were loaded by a desolvation cross-linking method and characterized by magnetic and physicochemical techniques. The synthesized nanoparticles were found to be spherical with an average diameter of 90 nm and zeta potential of about -49.4 mV.

The level of cell death involved in the pathway of apoptosis was measured to evaluate the synergistic effect of Au-SPIONs-mediated RF hyperthermia. MCF-7 and MDA-MB-231 human breast cancer cells were treated with different concentrations of Au-SPIONs. After incubation with NPs, the cells were exposed to RF waves (13.56 MHz; 100 W; 15 min). Cellular uptake of nanoparticles was confirmed qualitatively and quantitatively. The *in vitro* anti-tumor effect of the designed delivery vehicle on MCF7 and MDA-MB-231 human breast cancer cells was evaluated by cell viability assay. The results obtained from cell viability and apoptosis evaluation showed that NPs and radiofrequency (RF) had no significant effect when applied separately, while their combination had synergistic effects on cell viability percentage and doxorubicin release and the level of apoptosis induction. Experimental results revealed that it could significantly inhibit the proliferation of cancerous cells. Aptamer-functionalized iron-oxide gold nanoparticles improved cellular uptake and efficiency to breast cancer cells as compared to non-targeting nanoparticles because of the high affinity of mentioned aptamer toward the overexpressed nucleolin and targeting of HER2 and ERα on MCF7 and MDA-MB-231 cell surface. The obtained results showed that the use of aptamer-functionalized magneto-plasmonic NPs in the process of hyperthermia and radiofrequency (RF) of cancer holds a great promise to develop a new combinatorial breast cancer therapy strategy.



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Infrared-Emitting Nanoparticles for *in vivo* Early Tumor Detection

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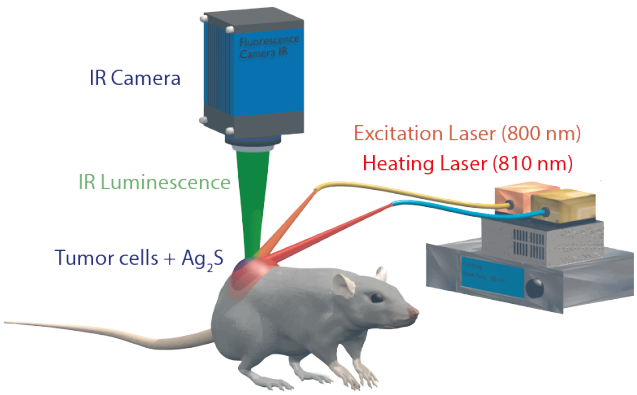
Development of technologies capable of early tumor detection is unquestionably demanded by physicians, as early diagnosis is the key to achieve more efficient and less invasive treatments with improved outcomes. At preclinical level, nanotechnology has already provided innovative solutions for tumor imaging and therapy, but it has failed to provide real early tumor diagnosis.

In this work, an infrared nanothermometry-based approach toward early tumor detection, based on changes produced in thermal relaxation dynamics of tissues is verified [1]. *In vivo* experiments demonstrate that detection of incipient tumors from their very onset is possible through monitoring changes in their thermal relaxation dynamics using Ag_2S infrared luminescent nanothermometers. Simultaneous study of the tumoral vasculature reveals that premature variation in thermal relaxation dynamics is a consequence of the interplay between tumor angiogenesis and necrosis during different tumor development stages.



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Supramolecular Assemblies For Nanomedicine

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Engineering of multifunctional vesicular (multi)compartments for mimicking cell functions is one promising approach for overcoming protein lack in organ tissues and human diseases [1]. These vesicular compartments have to fulfil various key characteristics (e.g. tuneable by external stimuli, controlling membrane functions for exchanging biomolecules, controlled release of biomolecules, retaining cargo inside of vesicular cavity), while multicompartments should also possess orthogonal-responsive membrane properties to control spatiotemporal and spatially separated biological pathways for establishing protocells [2]. Overall, this would result in, for example, establishment of next-generation therapeutics and bio-nanotechnology approaches.

This talk will present the use of pH-/T-responsive and crosslinked polymersomes and hollow capsules as versatile supramolecular tool for mimicking cell functions and for out-of-equilibrium approach to fabricate self-regulated on/off enzymatic nanoreactors. Besides established functional principles for polymeric vesicles and hollow capsules [3–7, unpublished results] recent progresses for the fabrication of artificial organelle with membrane/surface-integrated and/or lumen-integrated enzyme will be selected as well to being adaptable for mimicking cell functions (e.g. lysosome) and protocells.

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Dendrimers for Pharmaceutical Applications – Potential and Challenges

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The first very small dendrimers were synthesized in an academic laboratory and coined "cascade molecules" by Fritz Vögtle. This work did not raise much interest in academia but was noticed by industrial researchers who had been dabbling with similar ideas and had applications in mind. This is a natural consequence of how industry works; new products are developed with applications and markets in mind and it is also why many uses of dendrimers, still discussed today, in fact originated years back in companies such as Dow Chemicals in the US and DSM in the Netherlands. It is also important to think about the size of the market and what price the market is willing to pay for a new product. In the case of Dow Chemicals and DSM, they essentially helped giving birth to PAMAM- and PPI-dendrimers, made them commercially available, but left the field again. The market for dendrimers were too small and hyperbranched polymers can do many of the same things, but with much lower production costs. They did not have potential pharmaceutical applications in mind; it was a non-existing market within their timeframe and the volume of product would probably be too small compared to the investment.

The presentation will focus on the question: What are the most obvious obstacles in translating dendrimers from bench to clinical test site?

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Eosin Y and Rare-Earth-Doped Nanoparticles for Deep-Tissue Photodynamic Therapy

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Photodynamic therapy (PDT) consists in the generation of cytotoxic reactive oxygen species (ROS) by means of a photosensitizer (PS) and light [1]. The release of these oxygen species has many effects, but the most relevant for this work is cell death. PDT is a widely applied technique to treat cancer, because it is easy, selective and has fewer side effects for patients than other techniques. However, due to the attenuation that the light suffers through the tissues (skin, blood, etc.), PDT is only used to treat small tumors on or just under the skin or on the lining of internal organs or cavities.

The organic dye eosin Y is going to be used as PS. Eosin Y has good absorbance and emission in the visible range, and under excitation of 480 nm it generates singlet oxygen ($^1O_2$), this being the most important for PDT purposes. However, their biological applications are limited because they are not able to enter live cells, and can only pass through partially broken cell membranes (fixed cells). In order to solve this problem, we have attached eosin Y to rare-earth-doped nanoparticles via polyethylene glycol chains (PEG). These $NaGdF_4$: Nd^{3+}, Yb^{3+}, Tm^{3+} nanoparticles (NP's), when illuminated with light of 808 nm, are able to generate NIR light and also visible light via upconversion processes. The NP's have, therefore, two objectives, the first one: working as biological markers due to their near infrared (NIR) emissions, allowing us to work in the second biological window (II-BW). The second objective is directly related to the generation of ROS, because in order to excite the PS we are going to use the visible upconversion emission of the NP's around 480 nm from the de-excitation of various Nd^{3+} $^4G_{7/2}$ levels. We have shown that once NP and Eosin Y are linked, the resulting structure is able to bind to living cells and generate ROS, resulting in the death of cancer cells.

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Controlled Release of Chemotherapeutic Drug from a Stimuli Responsive Nanoparticle Incorporated in an Injectable Hydrogel for Treatment of Solid Tumours

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Nanotechnology approaches have been investigated for the treatment of different types of cancer, since they can be explored for localized and targeted therapy, reducing side effects and ineffective treatment. Mesoporous silica nanoparticles (MSNs) are inorganic nanoparticles with high surface area and loading capacity that can be surface modified to originate potent gated nanomedicines responsive to specific intracellular conditions, such as high concentration of reducing species [1, 2]. In addition, nanoparticles combined with hydrogels can be controlled and sustained released to improve the drug delivery system performance related to tolerability and toxicity. In this research, we developed a novel drug delivery system aiming to treat solid tumours, such as brain tumours, combining redox-responsive nanoparticles and injectable hydrogels.

MSNs were synthetized by the "liquid crystal templating" (LCT) method using a surfactant as a structure-directing agent. Once the surfactant template was removed, nanoparticles were loaded with either Safranin-O or Temozolomide (TMZ) and subsequently surface functionalised with polyethylene-glycol (PEG) through a disulphide bond forming a redox-responsive molecular gate. MSNs were characterized by different techniques such as powder x-ray diffraction (PXRD), Fourier-transform infrared spectroscopy (FTIR), dynamic light scattering (DLS), N2 adsorption-desorption and electron microscopy. The designed nanoparticles were incorporated into thermosensitive injectable hydrogel and their release was measured by Nanoparticle Tracking Analysis. Cytotoxicity of TMZ-loaded nanoparticles was tested on U87 glioblastoma cells.

MSNs with 1575 m2/g surface area and an average pore diameter of 2.7 nm were synthesized. After drug loading and functionalization with PEG, no changes in morphology were observed and the average size was 247 nm, as measured by DLS. TMZ-loaded nanoparticles without PEG gates released approximately 10 % of the estimated initial loading in 2 hours both in water and PBS pH 7.4. These drug-loaded nanoparticles were toxic to U87 cells decreasing their viability to less than 50 %. The PEG molecular gates controlled the release of both Safranin-O and TMZ payloads which were triggered to be released only after addition of a reducing agent (glutathione). Finally, MSNs incorporated in an injectable thermoresponsive hydrogel was sustained released up to 14 days and their size remained stable during the release.

A tuneable release profile of chemotherapeutic drug from nanoparticles was achieved through MSN surface functionalization with PEG. Nanoparticles delivered their cargo upon addition of reducing agents (GSH) that cleave the PEG-MSN attachment. The combination of nanoparticles and hydrogels originated a controlled release of nanoparticles that can be modified based on the hydrogel composition.

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Nanoparticle Localisation, Remote Thermometry and Hyperthermia Therapy Application Combined in a Preclinnical Magnetic Particle Imaging Scanner

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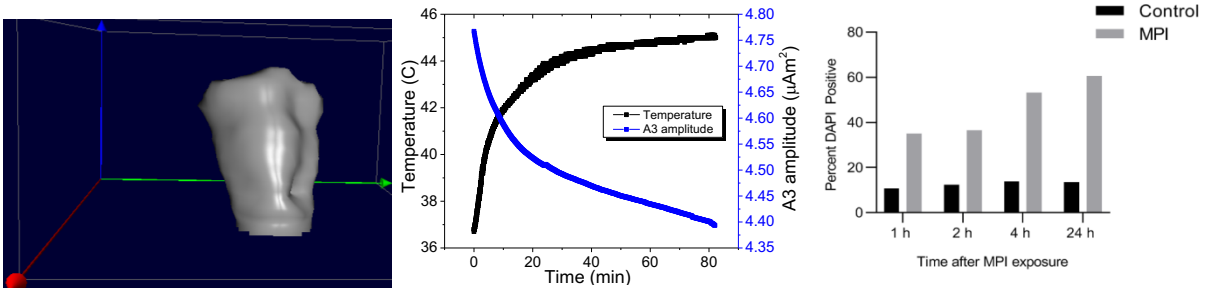
Localized hyperthermia actuated by magnetic nanoparticles (MNP) accumulated within tumor tissue has shown a great promise as a precise and minimally damaging cancer therapy. The technique has received much attention in recent years, with a concerted effort underway to improve aspects including MNP heating efficiency, administration routes and *in vivo* dosimetry. Here, we present a variety of experimental studies intended to demonstrate the opportunities offered by the novel biomedical imaging technique "Magnetic particle imaging" (MPI) in supporting the implementation of magnetic field hyperthermia (MFH) therapy.

We present experimental results showing how MPI employs spatially resolved dynamic magnetization measurements to reconstruct a 3D map of a nanoparticle distribution. In addition, it has been demonstrated that the imaging signal contains information about the local environment of nanoparticles, including their temperature. Furthermore, we present measurements proving that the complex arrangement of magnetic fields within the MPI scanner can be used to enhance the specific absorption rate of MNP. Using THP-1 leukemia monocytes, we present *in vitro* studies demonstrating effective thermoablation of a cancer cell population, using protracted exposure the fields present within the MPI scanner to induce cell death.

The study presents the capability of MPI to provide multi-functional support for MFH therapy by verifying location of MNP within the tumor, monitoring of the nanoparticle temperature for dosimetry control, and application of the therapeutic excitation field necessary to induce cell death within tumor tissue.



(a) Reconstructed MPI image of MNP in container with THP-1 cells. (b) Sample temperature and MPI signal amplitude change with prolonged exposure to MPI imaging fields. Correlation between sample heating and MPI signal demonstrates capability for remote thermometry using MPI (c) Flow cytometry showing 60% cell death 24 hours after MPI exposure for 80 minutes. Control sample received MNP but no MPI exposure.

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Study of New Synthetic Vectors for Cancer Gene Gherapy and Interference of Metastasis Process in Zebrafish Models

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Cancer is responsible for millions of deaths each year, mostly because of metastatic processes derived from the primary tumor [1]. Gene therapy, based on the regulation of gene expression, is a new therapy to cope with tumoral dissemination and metastasis formation [2]. One of the types of non-viral systems for gene therapy are nanosystems, which are biocompatible and biodegradable. Zebrafish can be used for *in vivo* studies.

Cationic nanosystems (NSC) were formulated through the ethanol injection method, and a plasmid encoding a fluorescent protein (mCherry) was associated. NSCs were characterized with ZetaSizer Nano ZS. *In vitro* assays were carried out in tumor cell lines (U-87 MG and MDA-MB-231). Result of cellular internalization assay was observed under the confocal microscope. Expression of mCherry was analyzed by fluorescence. *In vivo* assays were performed in 48 hours post fertilization zebrafish embryos. NSCs were incubated in the water or injected in the duct of Cuvier (DoC). NSCs interaction with cancer cells was tested in fish with U-87 MG cells.

The correct association of the plasmid to NSCs was proved on the view of changes in physicochemical properties of the NSCs (the size increases and the zeta potential after incubation of 5 µg of plasmid). *In vitro* tests have demonstrated the capacity of NSCs to interact and transfect tumor cells. Toxicity tests *in vivo* showed relevant differences among NSCs before and after plasmid association, most probably due to changes in the surface charge of NSCs (decrease of the zeta potential). NSCs were able to interact with zebrafish both after addition into water and injection into DoC. A stronger signal was observed for the plain NSCs (previous association of the plasmid). Importantly, we observed co-localization of the fluorescent signals corresponding to the NSCs and of the cancer cells xenotrasplanted in fish.

Zebrafish is a promising model for preclinical evaluation of innovative nanomedicines. Differences in toxicity, distribution, and interaction with cancer cells, were observed for our NSCs, mainly depending on their surface properties (before or after association of a plasmid). More experiments are currently undergoing to further explore the properties of our NSCs to interact with cancer cells in vivo and to mediate a therapeutic effect.

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Carbon Nanotubes are *Active-by-Design* Systems for Cancer Therapeutic Delivery

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Carbon nanotubes (CNTs) are nanomaterials which have drawn special attention in the field of nanobiotechnology. As other carbon allotropes, CNTs display an extraordinary capacity to capture biomolecules from the environment, acquiring different biological identities. Their unidimensional nature endows CNTs with unique properties *in vivo*. These nanofilaments can cross many different types of membranes, penetrating inside cells or tissues. More interestingly, their unique morphology and surface properties prompt their biomimetic interaction with intracellular biological nanofilaments – namely microtubules, actin and DNA – triggering interesting effects at the cellular level that deserve special attention when developing nano-vectors for anticancer therapies.

In the treatment of human cancer, multiple-drug resistance is a major problem. To circumvent this issue, clinicians combine several drugs. However, this strategy could backfire resulting in more toxic or ineffective treatments. We propose the use of multi-walled nanotubes (MWCNTs), in the design of active-by-design nanocarriers, attempting to enhance the effect of broadly used chemotherapies. MWCNTs display intrinsic properties against cancer interfering with microtubule dynamics and triggering anti-proliferative, anti-migratory and cytotoxic effects *in vitro* that result in tumour growth inhibition *in vivo*. Remarkably, these effects are maintained in tumours resistant to traditional microtubule-binding chemotherapies such as Taxol®. Moreover, our results demonstrate how MWCNTs significantly enhance the total antitumoural effect of the drug 5-Fluoracyl (50 % more effective) when delivered intratumourally coupled to MWCNTs both *in vitro* and *in vivo* in solid tumoral models. Our results demonstrate how using MWCNTs as anti-cancer drug delivery platforms is a promising approach to boost the efficacy of traditional chemotherapies, while considerably reducing chances of cancer cell resistance. All these intrinsic properties of CNTs have a huge potential if exploited in the development of nanodelivery platforms to treat cancer.

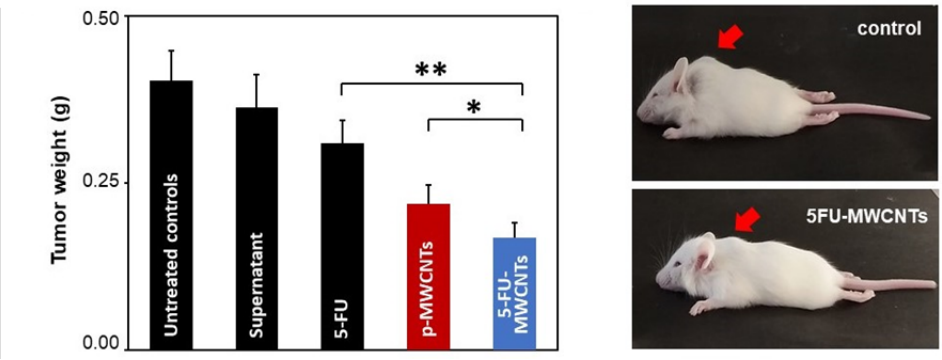

Figure 1. Statistical evaluation of the average melanoma tumoral weight 96 h post-treatment (single injection containing 2 µg of p-MWCNTs, 5-FU-MWCNTs or identical amounts of the 5-FU drug). 5-FU-MWCNTs trigger a statistically significant anti-tumoral effect respect to 5-FU injected locally ($t = 3.6$, $n = 75$, **= $t_{.99}$) or tumours treated with plain p-MWCNTs ($t = 1.31$, $n = 87$, * = $t_{.975}$). (right) Representative mouse littermates bearing solid melanoma tumours 96 h after a single intra-tumoral injection of a control medium or 5-FU-MWCNTs. Arrows point at the tumour location.

Poster presentations

Cabazitaxel-Loaded Poly(2-Ethylbutyl Cyanoacrylate) Nanoparticles Improve Treatment Efficacy in Patient-Derived Breast Cancer Xenograft

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The effect of poly(2-ethyl-butyl cyanoacrylate) nanoparticles (PEBCA NPs) containing cytotoxic drug cabazitaxel (CBZ) was studied in three breast cancer cell lines and one basal-like patient-derived xenograft model grown in mammary fat pad of immunodeficient mice [1].

NP-encapsulated CBZ had a much better efficacy than free drug in the basal-like patient-derived xenograft (Figure 1). Quantification of CBZ in mice plasma and selected tissue samples was performed by mass spectrometry. Drug encapsulated in NPs had a longer circulation time in blood. A three times higher drug concentration was measured in tumor tissue 24 h after injection of NPs with drug compared to the free drug. The tissue biodistribution obtained after 24 h using mass spectrometry analysis correlates well with biodistribution data obtained using IVIS® Spectrum *in vivo* imaging of fluorescent NPs, indicating that these data also are representative for the NP distribution. Infiltration of macrophages into a tumor tissue following injection of NP-encapsulated and free CBZ was estimated by immunohistochemistry. A higher infiltration of anti-tumorigenic versus pro-tumorigenic macrophages was found in tumors treated with the NPs. Tumor infiltration of pro-tumorigenic macrophages was four times lower when using nanoparticles containing CBZ than when using particles without drug, thus, we speculate that the very good therapeutic ef ficacy obtained with our CBZ-containing NPs may be due to their ability to reduce the level of pro-tumorigenic macrophages in the tumor.

Encapsulation of CBZ in PEBCA NPs seems promising for treatment of breast cancer.

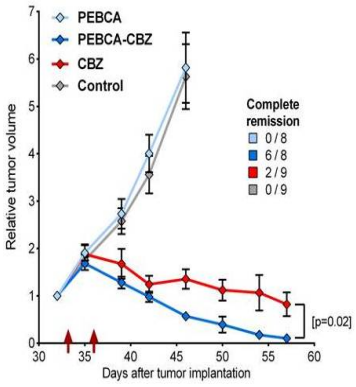

Fig. 1. Treatment efficacy in mice bearing MAS98.12 PDX breast tumors. Injections of 15 mg CBZ/kg were given twice (red arrows). Complete remission was obtained in 6 out of 8 tumors following injection of PEBCA-CBZ, whereas injection of the same dose of free CBZ resulted in complete remission of 2 out of 9 tumors. PEBCA NPs without drug gave the same tumor growth as in control mice injected saline. Body weight measurements demonstrated acceptable toxicity, and the improved efficacy is promising for lowering the systemic dose of the chemotherapy. Figure copied from [1].

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Fusser M., Øverbye A., Pandya A. D., Morch Y., Borgos S. E., Kildal W., Snipstad S., Sulheim E., Fleten K. G., Askautrud H. A., Engebraaten O., Flatmark K., Iversen T. G., Sandvig K., Skotland T., Mælandsmo G. M., J Contr Rel. **293**(2019), 183–192.

Synthesis and Physicochemical Properties of Multimodal Radiobioconjugate – Octreotide-PEG-^{198}AuNPs-PEG-DOX

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Over the last decade, one of the main causes of death in the world has been cancer. Surgery, chemotherapy and external radiotherapy are the most common conventional therapies currently applied. Systemic chemotherapy is often very limited due to serious side-effects to normal tissues. A consequence of these side effects is the use of suboptimal doses, which often result in therapeutic failure and development of drug resistance.

To increase the efficiency and selectivity of the therapy, in our project we propose a novel targeted brachytherapy applying radioactive gold nanoparticles (^{198}AuNPs), chemotherapeutic – doxorubicin and guiding vector – octreotide for the treatment of locally-advanced neuroendocrine cancers.

Synthesis of 30 nm radioactive gold nanoparticles was performed with the use of ^{198}Au precursor obtained in nuclear reactor in Świerk (Poland). For characterization of ^{198}AuNPs, non-radioactive ^{197}AuNPs synthesized according to the same procedures were used. TEM (transmission electron microscopy) and DLS (dynamic light scattering) techniques allowed to determine the mean size, particle size distribution and zeta potential. In order to avoid agglomeration and uptake by mononuclear phagocytes, the surface of nanoparticles was modified by polyethylene glycol (PEG, 5000 kDa). The PEG linker comprising the thiol group (HS) and the N-hydroxysuccinimide esters (NHS) at the ends was used for synthesis of PEG-DOX and PEG-Octreotide compounds. Finally, based on the strong affinity of sulphur to gold, synthesized PEG-DOX and PEG-Octreotide conjugates were spontaneously attached to AuNPs surface to form stable Au–S bond (Figure 1). The yield of the products was analyzed on the size exclusion column by HPLC technique. Promising *in vitro* studies on AR42J cancer cells are ongoing.

The obtained multimodal and multifunctional anticancer agent Octreotide-PEG-^{198}AuNPs-PEG-DOX could represent an attractive route for effective treatment of neuroendocrine cancers.

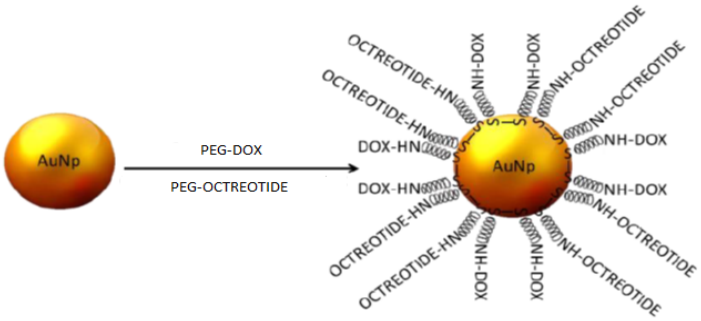

Figure 1. The structure of Octreotide-PEG-AuNPs-PEG-DOX bioconjugate

Novel Hybrid Iron Oxide@Gold Nanoflowers as a Potential Theranostic Agent for Cancer

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Hybridization of iron oxide nanoparticles with gold has attracted a much interest in recent nanomedicine related literature, due to a wide range of theranostic applications in cancer research that stem from the combination of their physicochemical properties.

On the one hand, maghemite iron oxide nanoflowers (IONfs) are superparamagnetic and have been reported for their potential in both magnetic resonance imaging (MRI) and magnetic hyperthermia treatment (HT) in cancer [1]. Gold nanoparticles, on the other hand, are biocompatible, can be easily functionalized with biomolecules and exhibit an intrinsic optical phenomenon of surface plasmon resonance (SPR) that allows for a variety of biomedical applications, such as computed tomography (CT) or confocal fluorescence imaging and photothermal therapy (PT) [2].

Herein, γ-Fe_2O_3 nanoflowers (IONfs) have been synthesized through a revised polyol method which produced a colloidally stable monodisperse aqueous ferrofluid. Following, for the synthesis of hybrid IONFs@gold (GIONfs), gold coating was achieved through the direct reduction of Au^{3+} on the surface of IONfs *via* a modified iterative seeding method using a strong reducing agent. For comparative purposes, plain gold nanoparticles (GNps) have been synthesized in aqueous medium. The isolated GIONfs, IONfs and GNps have been thoroughly characterized morphologically, structurally and for their magnetoplasmonic response. Biological evaluation has been conducted in vitro in different cancer and healthy cell lines in order to determine their cytotoxicity and their bioimaging potential.

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Influence of Synthesis Methods on Internalization of Fluorescent Gold Nanoparticles into Glioblastoma Stem-Like Cells

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Glioblastoma (GBM), the most common type of brain-born brain neoplasm, is an ultra-aggressive type of cancer with currently no satisfying treatment option available. GBM cells with stem cell properties (brain cancer stem-like cells, BTSCs) are thought to be responsible for initiation and propagation of the disease, as well as main contributors to the emergence of therapy resistance. Furthermore, the toxin clearing system of the blood-brain barrier (BBB) is believed to be responsible for the challenged delivery of pharmaceuticals to the anticipated target site. Novel anti-BTSC treatments with clinical applicability are of highest clinical and economic interest.

In this work, we developed a novel method to synthesize fluorescent gold nanoparticles as drug and gene delivery systems able to penetrate three-dimensional stem cell selected patient-derived GBM neurosphere systems *in vitro*. By using polyethylenimine (PEI) as a stabilizer and reducing agent, as well as fluorescein isothiocyanate (FITC) as a fluorescent marker, our fully *in-house* developed fluorescent gold nanoparticles (AuPEI-FITC NPs) with core sizes between 3 and 6 nm were obtained *via* a microwave-assisted reaction. Cytotoxicity, adsorption and internalization of AuPEI-FITC NPs into the BTSC cell lines JHH520, 407 and GBM1 were investigated using cellular growth assay and fluorescence-activated cell sorting (FACS) analysis. AuPEI-FITC NPs showed no apparent cytotoxicity and an uptake in cells of up to ~ 80 %. A differentiation between surface-bound and internalized AuPEI-FITC NPs was possible by quenching extracellular signals. This resulted in a maximal internalization degree of 61 %, which depends highly on the synthesis method of nanoparticles and the cell type tested. AuPEI-FITC NPs showed great potential as vehicles to transport DNA or drugs in most GBM cells due to their biological inertness and surface properties. Current efforts to attach novel discovered anti-BTSC pharmacologics to AuPEI-NPs are underway.

Targeting of Pancreatic Cancer by Albumin Nanoparticles

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Pancreatic cancer has an extremely poor prognosis with a median survival after diagnosis ranging between 2–8 months, being one of the most lethal cancers. New and more effective therapies are crucial to patients with pancreatic cancer improvement [1]. Drug resistance and numerous adverse side effects are huge barriers in conventional drugs against pancreatic cancer. In this way, medicinal plants are interesting sources of natural compounds with anticancer activity. In species belonging to the *Plectranthus* genus, their isolated compounds, such as the abietane diterpenoid Parvifloron D (PvD), have cytotoxic and antiproliferative activities against human tumor cells [2]. However, PvD is a very low water-soluble compound, being nanotechnology a promising efficient delivery system to this drug, giving the possibility of an enhanced drug solubility and targeted delivery with less adverse side effects [3].

Thus, the aim of this study was to optimize a nano-delivery system in order to target delivery PvD to pancreatic tumor cells. Albumin nanoparticles were produced through a desolvation method, which was optimized under different conditions and cytotoxicity assays on *Saccharomyces cerevisiae* model were made to study their potential cytotoxicity, showing no relevant results at the highest concentration tested. Using the optimized formulation, PvD was encapsulated (with a yield of 91.2 %) and nanoparticles were then characterized in terms of size (165 nm; PI 0.11), zeta potential (-7.88 mV) and morphology.

To target this nano-system to pancreatic cancer cells, a specific ligand was attached to PvD-loaded albumin nanoparticles surface and the antiproliferative effect was evaluated against two different human pancreatic tumor cell lines.

BSA nanoparticles were efficiently produced and PvD was successfully encapsulated. PvD-loaded BSA nanoparticles seem to be capable of being considered a suitable and promising carrier to delivery PvD in tumor site and to improve pancreatic cancer treatment.

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Gene Delivery Systems Based on Synthetic Lipid-Like 1.4-Dihydropyridine Core – Relationships of Self-Assembling and Physicochemical Properties

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Recent development of new non-viral vectors as DNA or drug delivery systems has resulted in elaboration of various nanopharmaceutical applications. Polyfunctional pyridinium derivatives on the 1.4-dihydropyridine (1.4-DHP) scaffold form liposomes and efficiently act as gene delivery agents [1, 2]. The influence of lipid head-groups [2], linker structure [3], remotion of cationic moieties [4] on transfection activity were studied.

The aim of the research includes: modification of substituents on the 1.4-DHP cycle; studies of biological activities and physicochemical properties; characterisation of liposomes formed by modified delivery agents; evaluation of interaction of liposomes formed by some synthetic lipids with phospholipid model membrane in Langmuir monolayer; clarification of structure-activity relationships.

A series of amphiphiles as putative gene delivery agents differing in substituents of 1.4-DHP core have been designed and synthesised. All amphiphiles possessed self-assembling properties, formation of nanoparticles with the average size 79–273 nm, which was dependant on the structure of the compound. 1.4-DHPs with modifications at the positions 2 and 6 or 4 of 1.4-DHP molecule showed the highest transfection activity of pEGFP-C1 plasmid DNA delivery into the BHK-21 cell line. It can be demonstrated that the structures of substituents at positions 2 and 6 of 1.4-DHP molecule are important for radical scavenging properties. The buffering capacity of studied N-unsubstituted 1.4-DHPs were in the pH range of 6.8–8.8. Liposome interaction with the monolayer is influenced by the composition and surface pressure of the monolayer, temperature, composition and pH of the sub-phase, as well as modification of the surface of the liposomes. The obtained data suggest that a biomembrane model – Langmuir monolayer can be used to study liposome interactions with different model membranes.

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Synthesis and Pharmaceutical Applications of Polyhydroxy-Dendrons Containing an Amino Focal Group for Conjugation to Biological Active Compounds

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Interactions of carbohydrates with lectins are involved in crucial intercellular recognition events and this has led to a search for potential ligands. The concept of enhanced-affinity multivalent binding has inspired synthesis of compounds with multi-carbohydrate peripheries, which has allowed to report on new low generation glycodendrimeric compounds and a structural investigation of their interactions with human galectin-7 (hGAl-7), a lectin involved in tumour growth [1], as well as thermodynamic studies on their binding to peanut agglutinin (PNA) [2]. Modifications of a dendron containing polyhydroxy or carbohydrate groups have been described as potentially biologically useful agents in three areas of interest:

1. Introduction of a PEG-biotin spacer for bioconjugation to avidin-modified polymersomes, in order to establish an ECM docking station on cell surfaces and artificial cell mimics for carrying out ECM enzymatic reactions [3, 4]. Polymersomes have protein-repellent properties while, by targeting hGAl-7, it will be investigated whether receptors can recognize it specifically.

2. Liposomes are promising nanosystems for targeted drug delivery; therefore, their versatility to attach various surface moieties has been exploited [5]. Thus, DPPC-phospholipid membranes were mixed with molecules formed from the coupling of glycodendron blocks with 4.4'-((1.4-phenylenebis(methylene))bis(oxy))dibenzoic acid. DSC evaluation showed that this approach allows the development of stable liposomal systems with surface glycodendrimers.

3. Effect of a hybrid molecule formed by conjugation of an antioxidant moiety to an amino building block grafted with polyhydroxy groups on proteasome function has been investigated. Results suggest potential proteasome activation through transcriptional up-regulation of its subunits. Results from treatment of HFL-1 human primary fibroblasts and wild type *C. elegans* with this hybrid molecule indicate that it may be used in potential anti-aging strategy [6].

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Influence of Phosphonium Carbosilane Dendrimers (CS-DDMs) on Crythrocytes

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Herein we present novel types of generation 1–3 carbosilane dendrimers (CS-DDMs) modified with various types of peripheral phosphonium groups [1]. Phosphonium peripheral groups were synthesized by coupling of an appropriate phosphine to iodopropyl-terminated dendrimers. To manifest their potential in biomedical applications, we have performed comparative *in vitro* toxicity study on erythrocytes. Two methods were used to determine the effect of dendrimers on red blood cells – hemolysis and microscope observation. Dendrimers have been monitored in several concentrations range 1 □M, 5 □M, 10 □M and 50 □M as well as different incubation times 1 h, 6 h, 12 h and 24 h.

These experiments revealed that all kinds of investigated CS-DDMs have influence on red blood cells and induce their hemolysis process. Percentage of hemolysis was related with dendrimer generation and concentration, time of incubation and functional groups. It has been shown that with the growing dendrimer generation, concentration and time of incubation toxic effect was stronger. The type of central phosphonium moiety functionalization has significant influence on extent of toxic effect on erythrocytes membrane. The CS-DDMs with more hydrophobic PBu_3 and $P(Ph)_3$ peripheral groups induced higher hemolysis process. It was also observed that dendrimers with hydrophobic functional groups after hemoglobin release induced its aggregation process which occurred as large brown aggregates observed in a solution (even by the unaided eye). It has been shown that during the time aggregates were enlarged (microscopy observation) while in solution concentration of hemoglobin was significantly decreased. Dendrimers with PMe_3 and $P(C_6H_4\text{-}OMe)_3$ peripheral groups caused less hemolysis and aggregation process was not observed.

Generally, CS-DDMs with more hydrophilic functional groups due to their lower toxic effect can be good candidates as drug delivery systems.

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Graphene Functionalised with Arginine and Proline as Drug Delivery System

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Glioblastoma multiforme (GBM) is the most aggressive and lethal subtype of brain tumour. The prognosis for patients is poor; the median patient survival after diagnosis is approximately one year. Previously, it has been demonstrated that graphene has anticancer properties in experiments *in vitro* with glioblastoma multiforme (GBM) cells and with tumour tissue cultured *in vivo* [1]. However, because of the tendency for agglomeration of nanoparticles, toxicity to cells and tissue could be diminished. In the present study, it has been hypothesised that functionalisation of graphene with arginine or proline might counteract graphene agglomeration and increase the area of graphene activities. Moreover, proline and arginine – amino acids preferentially located in different compartments in the cell and extracellular matrix (ECM) can anchor graphene to the target tissue of the tumour. The experiments were performed *in vitro* with GBM U87 cells and *in ovo* with GBM tumour, cultured on chicken embryo chorioallantoic membrane. The measurements included cell morphology, mortality, viability, tumour morphology, histology and gene expression. The cells and tumours were treated with reduced graphene (rGO) and rGO functionalised with arginine (rGO+Arg) or proline (rGO+Pro).

The results confirmed the anticancer effect of graphene on the GBM cells and tumour tissue. rGO increased expression of *TP53* gene. Moreover, after functionalisation with amino acids, nanoparticles were distributed more specifically – rGO+Pro were mainly deposited in ECM, while rGO+Arg within cells. Graphene flakes were less agglomerated. Arginine but not proline, enhanced the anticancer activity of rGO at the molecular level. The molecule of rGO+Arg increased expression of *TP53* gene but did not increase mRNA expression of *MDM2* and the ratio of *MDM2/TP53* was diminished in the tumour, suggesting that arginine may block MDM2 expression. Furthermore, rGO+Arg molecule did not diminish *COX6* and *CASP3* mRNA expression, increased by rGO treatment, indicating that the pro-apoptotic character of rGO was not reduced by arginine functionalization. The expression of *NQO1* gene, being a strong protector of p53 protein in the tumour tissue, was highly increased. The results indicate that the interacting action of rGO and arginine has potentials in GBM therapy.

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Scaffolds of Carbon Nanoparticles Can Mimic a Muscle Cell Niche for Regeneration of Muscle Tissue after Oncological Deficits

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Oncological surgeries are often associated with resection of muscle tissue, hence the rebuilding the tissue is therapeutically crucial. The environment of stem cells is called stem cell niche, a key factor for their further proliferation and differentiation. Standard methods of cell culturing include only an effect of the two-dimensional substrate surface. Creation of the 3D structure that allows for culturing of muscle cells from their progenitor cells could be essential for *in vitro* cultivation of muscle implants. Carbon allotropes, as a 3D scaffold of the niche, can create perfect environment for cell growth, particularly, because it is a highly biocompatible material [1].

Carbon scaffolds were prepared by layer placement and desiccation of the colloids of nanoparticles of diamond (ND), fullerenes (F60), nanotubes (NT), nanotubes OH (NTOH), nanotubes COOH (NTCOOH) and graphene oxide (GO). Mesenchymal stem cells were collected from the hind limb bud of 7-day-old chicken embryos. Morphology of mesenchymal and muscle cells was visualized and expression of the mRNA of chosen proteins was measured.

Interaction of nano-scaffolds with cells differed and the number and state of development of cells were influenced by carbon allotropes. For stem cells, the most neutral was ND based scaffold. The scaffold, prepared from F60 was the most colonised by cells; moreover, it stimulated cell proliferation. The scaffold constructed from NTCOOH was also well settled by cells, better than scaffold with NT and NTOH. GO scaffold stimulated differentiation of muscle stem cells and creation of the muscle tissue. mRNA expression of muscle cells colonised in GO scaffold clearly showed increased expression of MyoD-marker of differentiated muscle cells.

Results indicate that nano-scaffolds, depending on carbon allotropes, influence behaviour and morphology of muscle stem cells; moreover, their functionalisation change bio-function of scaffolds and stem cells number and morphology. Consequently, 3D nano-scaffolds can mimic cell niche for regeneration of muscle tissue after oncological deficits.

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Peptide-Targeted Polymersomes for Cancer Therapy and Detection

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Cancer is the second leading cause of deaths worldwide [1] and the mortality is expected to be rising in the future [2]. Surgery and radiation therapy successfully eliminate local and easily accessible tumors, but the disease spread all over the body can be controlled only by systemic chemotherapy. However, for conventional chemotherapeutics, poor bioaccessibility, penetration, high off-target toxicity, and side effects due to exposure of normal cells to cytotoxic compounds remain unsolved challenges. These problems can be alleviated by development of drug delivering nanoparticles (NPs) that specifically accumulate in tumors thus reducing systemic cytotoxicity [3]. For this purpose, drug nanocarriers have stealth properties thus prolonging circulation half-life in the bloodstream letting drug-loaded nanocarriers to reach the tumor [4, 5]. In order to enhance selectivity and accumulation of nanocarriers in target tissue, they can be functionalized with ligands having affinity to tumor tissue, such as antibodies and tumor homing peptides among others [6, 7]. Moreover, the NPs can be engineered to contain not only drugs and targeting ligands but also agents that allow their tracking for early and accurate cancer diagnosis [3].

We developed new peptide-targeted polymeric nanovesicles (polymersomes, PS) that specifically target, penetrate and deliver antitumor therapeutics to tumor cells *in vitro*, and specifically accumulate in triple negative breast (TNBC) tumors *in vivo*. PS were functionalized with the tumor-penetrating CendR peptides that bind to specific receptors overexpressed in tumors. The treatment of PPC-1 cancer cells (expressing the RPAR receptor) with doxorubicin-loaded RPAR-targeted PS significantly reduced the cell viability compared with the treatment with nontargeted PS. Fluorescent-labeled PS accumulated into triple negative breast tumor in mice and this accumulation was enhanced by functionalizing the PS with the tumor penetrating peptides (40 % of increase using LinTT1 and 26 % using RPAR). Our results indicate the suitability of peptide-targeted PS for detection and treatment of TNBC.

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In Vivo Biodistribution of Protein Nanoparticles for Cxcr4$^+$ Cell-Specific Targeting in Metastatic Colorectal Cancer Model

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Metastasis is the main cause of death in colorectal cancer (CRC) patients. CXCR4 receptor is overexpressed in metastatic stem cells (MetSCs) and associated with poor survival and recurrence in CRC patients. Targeted drug delivery to MetSCs promises to increase drug uptake by cancer cells and prevent metastasis while reducing systemic toxicity; thus, improving current therapies.

Protein nanoparticle T22-GFP-H6 was developed and physico-chemically characterized as a targeting vector that includes a ligand CXCR4 (T22), which demonstrated selective internalization in CXCR4$^+$ CRC cells *in vitro*. We also generated CXCR4 overexpressing subcutaneous and orthotopic SW1417 CRC models. *In-vivo* biodistribution assays were performed in both models after i.v. injection using a 20–500 μg dose range. a highly selective T22-GFP-H6 uptake in tumor tissue was observed, with a 90 % of total fluorescence accumulated in tumor tissue (confirmed by anti-GFP IHC), with insignificant distribution to normal tissues, including liver. The administrated doses did not induce any sign of toxicity or histological alteration in animals.

It was also demonstrated that SC injection of SDF1-alpha inhibits T22-GFP-H6 internalization, demonstrating CXCR4-dependent accumulation in tumor cells. In addition, T22-GFP-H6 biodistribution assays in the metastatic model also showed nanoparticle accumulation in the primary tumor and all macro or micro-mestatatic foci lasting longer than 24 h, and its internalization in CXCR4$^+$ cells, again with lack of toxicity on normal organs. Once, protein-only T22-GFP-H6 nanoparticle was chemically conjugated to an antitumor drug. We expect to demonstrate in the CXCR4$^+$ SW1417 CRC model its capacity for selectively deliver the drug into the cytosol of CXCR4$^+$ metastatic stem cells, leading to antimetastatic activity. Therefore, our aim is to generate a new drug that improves current therapy, by eliminating cells responsible for cancer dissemination, leading to lower disease load while maintaining the toxicity low.

Synthesis, Characterisation and Molecular Dynamics of Two Peptide Dendrimers Consisting of Lys2Gly and Lys2Lys Repeating Units for Drug and Gene Delivery

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Dendrimers are regularly branched almost monodisperse macromolecules with a well-defined structure. Poly-L-lysine and more general peptide dendrimers are good candidates for many biomedical applications due to their high biocompatibility and relatively low toxicity in comparison with many other dendrimers [1]. Size, anisotropy, local internal structure and orientational mobility of terminal, side and main chain dendrimer groups are important characteristics for drug and gene delivery and for other specific applications [2–14].

We present here synthesis, characterization and molecular dynamics of two new lysine-based peptide dendrimers (Lys2Gly and Lys2Lys) with two additional linear aminoacid residues (2Gly or 2Lys) inserted between each neighboring branching points of conventional lysine dendrimer. We also study here temperature dependences of relaxation times of terminal, side and main chain groups in the temperature range from 283 to 343 K.

We have shown that temperature dependences of relaxation times and spin-lattice relaxation time T_{1H} of CH_2 groups of both dendrimers (Lys2Gly without and Lys2Lys with side fragments) are very close to each other despite big difference of densities of atoms inside these dendrimers. This work is supported by RFBR grant 19-03-00715.

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Selective Delivery of T22-PE24-H6 to CXCR4$^+$ Diffuse Large B-cell Lymphoma Cells Leading to Potent Antineoplastic Effect in Disseminated Mouse Model

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Nowadays novel therapeutic strategies are urgently needed to reduce relapse rates and enhance survival of Diffuse Large B-Cell Lymphoma (DLBCL) patients. Delivery of toxins to malignant clones that drive tumor development is a novel approach that yielded encouraging results in several cancer types. Cell selectivity is achieved by developing vectors able to transport and deliver drugs to tumor cells that overexpress specific receptors. Here, we have evaluated antitumor effect of a novel polypeptidic nanoparticle targeting the CXCR4 receptor (by incorporating the T22 ligand), which is overexpressed in 50 % of malignant DLBCL B-cell lymphocytes compared to normal B-cells. Moreover, CXCR4$^+$ DLBCL cells are responsible for lymphoma cells dissemination, relapse and resistance to R-CHOP. In this regard, T22-PE24-H6 polypeptidic protein acts as a self-assembling CXCR4 cell-targeted nanoparticle that delivers the catalytic domain of Pseudomonas Aeruginosa (PE24) upon cell internalization into DLBCL cells with CXCR4 overexpression. PE24 is able to perform ADP-ribosylation of the elongation factor 2 (EF-2), producing an irreversible inhibition of protein synthesis and cell death.

The presented study aimed to evaluate selective antineoplastic effect of the T22-PE24-H6 polypeptidic nanoparticle to CXCR4$^+$ DLBCL cells both *in vitro* and, most importantly, in a disseminated mouse model, which replicates organ involvement observed in DLBCL patients.

Performing cell viability assays, T22-PE24-H6 demonstrated *in vitro* antineoplastic effect in different CXCR4$^+$ DLBCL cell lines and no effect in CXCR4$^-$ DLBCL cell line. The most sensitive cell line to the toxin was Toledo and, thus, we chose it for the *in vivo* experiments. In addition, the specificity of the nanoparticle entrance through the CXCR4 receptor was also demonstrated by performing competition assays with the CXCR4 antagonist (AMD3100). In a disseminated mouse model intravenously injected with CXCR4$^+$ Toledo-Luci cells, T22-PE24-H6 treated mice (5 µg of intravenous T22-PE24-H6, 13 doses, 3 times/week) showed a highly significant reduction of bioluminescence signal (BLI) compared to the buffer-treated mice. Importantly, this significant reduction of BLI was displayed in organs clinically affected by DLBCL cells (lymph nodes and bone marrow). Finally, we did not observe any differences in the mice body weight neither did we observe histopathological alterations in any non-affected DLBCL organ.

T22-PE24-H6 promises to become an effective alternative to treat refractory or relapsed CXCR4$^+$ DLBCL patients without systemic toxicity.

Affinity-Targeted Silver Nanoparticles as a Research Tool and a Drug Carrier

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We have developed silver nanoparticles (AgNPs) as a model cargo to study tumor homing peptide-mediated targeting and cellular/tissue interactions of AgNPs *in vitro* and *in vivo*. AgNPs loaded with fluorescent dyes can be tracked in cells and tissues by ultrasensitive optical imaging, as silver cores of the AgNPs plasmonically enhance the fluorescent signal [1]. To allow quantitative internally-controlled cellular uptake and tissue biodistribution studies using AgNPs, we have developed isotopically barcoded AgNPs and optimized their detection by inductively coupled plasma mass spectrometry (ICP-MS) and laser ablation ICP-MS. This approach allows ultrasensitive parallel auditioning of peptide-guided and control AgNPs in the same biological test systems [2]. Furthermore, peptide-targeted AgNPs can serve as a carrier for cytotoxic payloads to receptor-positive cancer cells [3]. We show that AgNPs loaded with a potent anticancer drug, monomethyl auristatin E (MMAE), and targeted with Neuropilin-1-targeting C-end Rule peptide, RPARPAR, accumulate in prostate cancer cells overexpressing the receptor protein and cause selective toxicity in these cells. Importantly, the specific cytotoxic activity of RPARPAR-MMAE-AgNPs (vs. non-targeted MMAE-AgNPs) can be potentiated by dissolution of the extracellular nanoparticles by a mild biocompatible etching solution [1].

The studies suggest that AgNP platform can be used for quantitative nanoparticle biodistribution studies and as an anticancer drug carrier, and that elimination of extracellular AgNPs by exposure to etching solution can be used as an endocytosis research tool and as a means to improve the therapeutic index of AgNPs loaded with anticancer payloads.

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Toxic Effect of Nanoparticles, N-Acetylglucosamine Polymer and Protein Extract on Hepatic Stem Cells

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Liver stem cells represent useful tool for studying toxicity and morphology changes after nanoparticles (NP) treatment and can be an alternative approach to liver transplantation [1]. Chicken embryo hepatocytes are difficult to maintain in *in vitro* culture and require optimization of passage conditions due to lack of heterotypic interactions. The boundaries of hepatocyte islands, in the vicinity of fibroblasts, show increased heterotypic cell–cell interactions and high proliferation [2]. Colony size have influence on variations in cell signalling, growth factors release, extracellular matrix deposition and protein production.

Suspension of 10-day-old chicken embryo liver cells was prepared by overnight trypsinization at 4 °C. The isolated cells were seeded in DMEM supplemented with 10 % Fetal Bovine Serum and 1 % penicillin:streptomycin. Two experiments were performed: 1) optimization of passage conditions, 2) influence of NP on morphology and cell viability (XTT assay). To assess the effect of cells co-culturing, liver cells and HS-5 cells were cultured (ATCC, CRL-11882). As a control, hepatocytes and HS-5 cells were cultured alone. After 3 days, cells were collected by scraping, fixed with 4 % paraformaldehyde, and stained with hematoxylin/eosin. In the second experiment, after cells adhesion to 96-wells microplates, NP were added to the culture: Ag, Au and MnO (5 µg/ml); Pt (1 µg/ml); GO and C_{60} (10 µg/ml); chitosan and protein extract (1 %).

The toxicity of the NP declined in the order: Ag> Pt >Au> GO> chitosan> C_{60}> MnO> protein extract. When hepatocytes that proliferated in co-cultures with HS-5 were harvested and further subcultured, their behavior was similar, i.e. grew in clusters surrounded by HS-5 cells. Generally, their morphology was similar to that of freshly isolated cells.

All other tested NP appeared to be less toxic than Ag-NP, also observed by Faedmaleki et al. (2014). After addition of C_{60} and MnO, colonies increased in size and the number of cells per colony appeared to increase as well. Thus, increasing colony size may be in correlation with improvement in hepatocellular functions. Probably heterotypic cell-cell interactions between liver cells and HS-5 resulted in normal passage of the cells. These results suggest that hepatocytes need heterotypic cell-cell interactions for longer culturing *in vitro*.

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Reduced Graphene Oxides Change Expression of Cell Receptors and Voltage-Dependent Ion Channel Genes of Glioblastoma Multiforme

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Graphene (GN) and reduced graphene oxides (rGOs) show anticancer properties in glioblastoma multiforme (GBM) cells *in vitro* and in tumors *in vivo*. We compared anti-tumor effects of rGOs with different oxygen contents with those of GN and determined characteristics of rGOs useful in anti-glioblastoma therapy using the U87 glioblastoma line.

We analysed damage of cell membrane using an LDH assay. We also evaluated DCFDA-Cellular Reactive Oxygen Species Detection Assay Kit and Cellular Membrane Potential Assay Kit for detection reactive oxygen species and change in cell membrane charge. To study the changes related to voltage-dependent ion channels, the following genes were analysed: *clcn3, clcn6, cacna1b, cacna1d, nalcn, kcnj10.*

The results showed no significant changes in cell membrane damage. However, a statistically significant increase in ROS in cells was observed in the first contact of flakes with glioblastoma cells. The analysis of gene expression of voltage-dependent ion channels showed a statistically significant increase in expression of *clcn3* and *nalcn*, especially in the rGO/ATS and rGO/TUD treated groups. A statistically significant decrease in *cacna1b* expression was also observed in the same group.

It can be concluded that the studied graphene forms strongly influence gene expression of ion channels, the effect of which is strongly related to apoptosis, migration and invasiveness.

MRI-Guided Focused Ultrasound Robotic System for Preclinical Use

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An MRI-guided focused ultrasound (MRgFUS) system was developed that can be used for preclinical studies in mice.

A single element spherically focused transducer of 4 cm diameter, focusing at 6.5 cm and operating at 2.4 MHz was used. The positioning device incorporates only MRI compatible materials. The propagation of ultrasound is a bottom to top approach. The robotic system includes 2 linear axes (6 cm range each with 0.1 mm maximum motion error).

The system was tested successfully in agar/silica/evaporated milk phantom for various tasks such as MR compatibility, motion accuracy and functionality.

This simple and functional design can be a useful research tool for preclinical work on mice. This system has the potential to be marketed as a cost-effective solution for performing experiments in mice. The device can be used in synergy with therapeutic ultrasound and nanodrugs. With some modifications this device can be also used for a 9.4 T MRI scanner.

Figure 1: Schematic of the developed robotic system.

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Combination of Doxorrubicin and Dendritic Molecules agains Breast and Prostate Cancer

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Approximately 1 out of 6 deaths in the word is due to cancer with about 9.6 millions of deaths in 2018. Most prevalent cancers are: lung, breast, colorectal and prostate cancer. From them, androgen-independent prostate cancer and triple negative breast cancer increase death ratios due to low effectiveness of treatments and increased frequency of metastasis.

Research on cancer combination therapies has widely increased during last decades, looking for therapies with low secondary effects and no resistance production [1, 2].

In this sense, nanotechnology and particularly dendritic nanosystems, being extensively studied for biomedical applications such as cancer diagnosis, drug delivery and targeting, gene delivery… etc. [3, 4], are arising as promising tools for these combination therapies improving the way of action of tradicional antitumoral drugs.

This work is focused in the combination of different carbosilane dendritic molecules and dendronized gold nanoparticle with commercial drugs, such as doxorubicine, against HCC triple negative breast cancer cell line and PC3 androgen-independent prostate cancer cell line (Figure 1). Data show that carbosilane dendritic systems can improve the antitumoral activity of traditional drugs when used together in combination therapy.

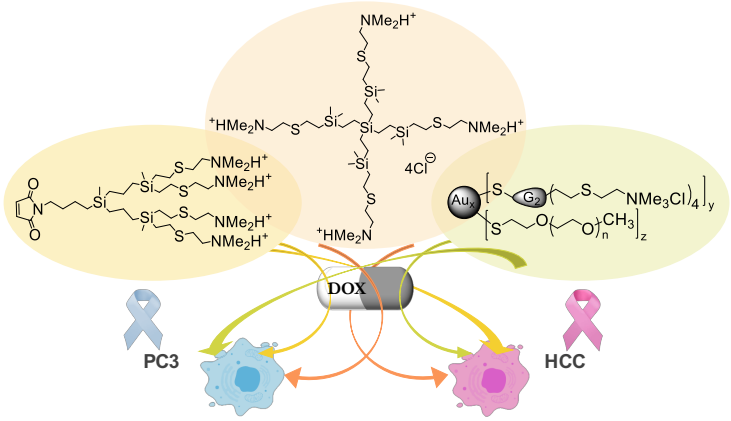

Figure 1. Schematic representation of performed work

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Evaluation of Cytotoxic Effects of Complexes of Cucurbit[7]uril and Antitumor Pt(II)-Derived Drugs

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We studied cytotoxic effects of complexes of Pt(II)-derived drugs (carboplatin, oxaliplatin) with cucurbit[7]uril (CB[7]) on primary cell culture (peripheral blood mononuclear cells, PBMCs) and cell lines (B16, K562).

Cells were cultivated in a 5 % CO2 humidified atmosphere at 37 °C for 48 h (B16 and K562 cells) or 72 h (PBMCs) in the presence of CB[7]-oxaliplatin complex or carboplatin in mixture with CB[7] (1:1) in different concentrations (0.1, 0.2, and 0.3 mM). Cytotoxic effect was then evaluated using the MTT assay or the WST assay. To assess cell proliferation by flow cytometry, cells were stained with 5.6-carboxyfluorescein diacetate succinimidyl ester (CFSE) before cultivating.

CB[7]-oxaliplatin complex demonstrated greater cytotoxic effect on tumor cells (B16, K562) compared to oxaliplatin. Although carboplatin does not form a stable inclusion complex with CB[7], we found that the addition of CB[7] affects the biological properties of carboplatin. Thus, carboplatin with CB[7] had higher antitumor effect on murine B16 melanoma cell line than carboplatin. We hypothesized that enhancement of antitumor effect of carboplatin with CB[7] can be shown on murine melanoma cell line, while hematopoietic stem cells-derived tumors may have higher viability in similar conditions. When we used PBMCs from patients with B-cell lymphomas (60.3 ± 5.11 % of cells in the sample were tumor cells), it was demonstrated that carboplatin with CB [7] (1:1) weakly reduced viability of patients` PBMCs than carboplatin alone. Therefore, CB7 probably reduced the toxic effect of carboplatin on healthy immune cells, as well as on tumor cells developed from immunocompetent cells. The complexation of platinum compounds with CB[7] did not enhance the cytotoxic effect on PBMCs also.

Our findings suggest that CB[7] can be a prospective nanocarrier for increasing antitumor properties of Pt(II)-derived drugs.

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Amphiphilic Triazine-Carbosilane Dendrons Containing Chargeable and Non-Chargeable Interior

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Supramolecular constructions are considered carriers for anti-cancer drugs due to combination of physico-chemical and biological properties. In particular, amphiphilic dendritic molecules forming associates of given topology are promising building blocks for such assemblies.

Recently, we have reported a new class of functional dendritic species – amphiphilic triazine-carbosilane dendrons (Figure). The presence of branched hydrophobic unit drives self-assembly of dendrons in water medium into bilayer supramolecular constructions – dendrimerosomes. In this work, we explore the impact of the dendron interior on its self-assembly. Two series of dendrons consisting of hydrophobic triazine unit and carbosilane dendrons bound through either chargeable (piperazine) or non-chargeable (aminohexanoyl) linkers have been prepared (Figure). Both types of dendrons form stable associates - dendrimerosomes. The associates are pH-sensitive and reorganize at slightly acidic pH.

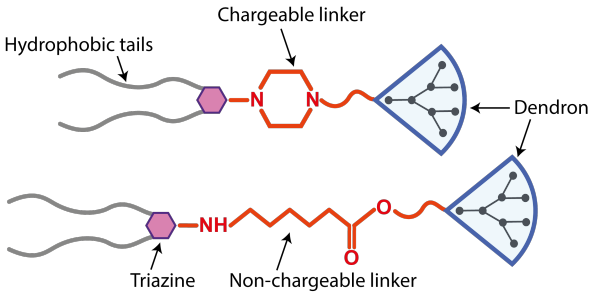

Figure. Sketches of triazine-carbosilane dendrons with chargeable and non-chargeable interior

Dendrimerosomes have been shown to encapsulate chemodrugs (DOX, methotrexate) efficiently as well as bind anti-cancer nucleic acids (Mcl-1 siRNA). Our findings suggest that amphiphilic triazine-carbosilane dendrons are promising carriers for anti-cancer therapeutics.

Acknowledgements: This study was supported by RFBR grant 18-33-20109, by MINECO grant CTQ-2017-85224-P, by the grant of the President of RF MK-2278.2019.4.

Use of Selected Carbon Nanoparticles as Melittin Carriers for MCF-7 and MDA-MB-231 Breast Cancer Cells

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According to World Health Organization, breast cancer is the most frequent cancer among women, impacting 2.1 million women each year, and also causes the greatest number of cancer-related deaths among women. The intensive development of medicine has provided us with many types of treatment for this type of cancer. Despite this, many women and men die of this type of cancer. Therefore, new methods of treatment are constantly sought, often with the use of natural sources.

Melittin (MEL) is the main biologically active component of bee venom, constituting for approximately 40–50 %. It is linear consisting of 26 amino acid peptide with water-soluble and amphipathic properties [1]. Many studies show the biological effects of melittin as antibacterial, antiviral, antifungal, anti-parasitic and suggest that melittin has non-selective cytolytic activity, acts physically and chemically disrupts all prokaryotic and eukaryotic cell membranes [2].

Nanotechnology is an intensively developing multifaceted field, which is used in more and more new areas of science. Recently, nanomaterials and nanoparticles have found many applications in the field of nanomedicine, particularly in drug delivery systems. Nanoparticles are promising carriers for therapeutic agents due to the mechanism of their uptake by cells – **internalization** [3]. Is another term for endocytosis, in which molecules such as proteins are engulfed by cell membrane and transport into the cell. The examined nanoparticles did not show strong toxicity but effectively deregulated cell migration. ND was effectively taken up by cells, whereas nGO and NG strongly interacted with the cell surface.

Recently, influence of melittin on regulation of apoptosis in many types of cancer has been extensively studied. It was observed that melittin activates caspases in leukemia, melanoma, prostate and cervical cells. But the effect is different for different cell types. Melittin exhibits necrotic cytotoxicity in gastrointestinal cells. Therefore, it is important that for each type of cancer the effect of this peptide is checked. The challenge in using it is the lytic activity of melittin in relation to all biological membranes that can be manipulated using different particles. The aim of the study is to determine the use of nanoparticles as melittin carriers for breast cancer cells by studying toxicity, dose dependence and activation of cell death.

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Amphiphilic Dendritic Compounds for Drug Delivery Purposes

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Recently, a high biomedical potential of phosphonium dendrimers was discovered in our group [1, 2]. Following that, the work aims on preparation of amphiphilic of dendritic substances such as **1** (Figure 1) which could serve as drug delivery systems in forms of supramolecular objects (e.g. liposomes). Primarily, molecules bearing phosphonium groups as a polar domain are prepared; however, designed synthesis is also used for preparation of non-ionic amphiphiles bearing hydroxyl groups (e.g. **3** (Figure 1)). A main goal is to be able to assemble supramolecular structures which could transport defined siRNA (short interfering RNA) to cytoplasm but other applications are possible as well.

Parallelly, the work focuses on preparation of compounds such as **2** or **4** –unsymmetrical analogues of substances **1** and **3** with one terminal functional group. Their purpose is to connect a fluorescent tag on one of their lipophilic arms and make liposomes or other supramolecular objects from mixtures of symmetrical and tagged unsymmetrical molecules. This should enable easy detection of the assembled supramolecular objects in living systems.

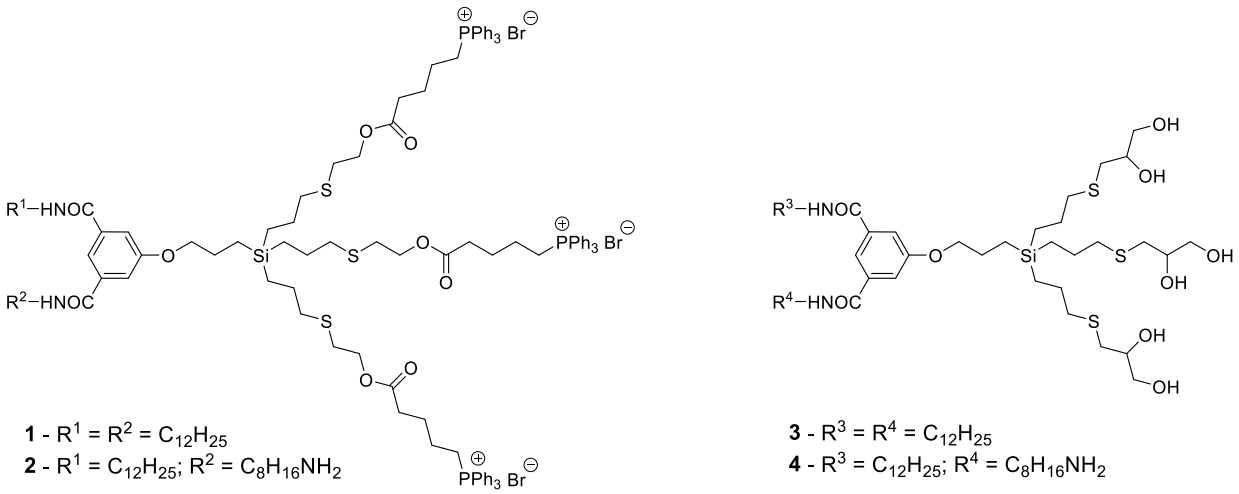

Figure 1. Amphiphilic dendritic molecules which could serve as drug delivery systems

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Nanoparticles for Effective Treatment of Cervical Cancer in HIV-1 Infected Women in Female Reproductive Tract

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Cancer of cervix is caused by an aberrant cell growth that acquires an ability to invade other body parts. It has also been reported to be the second most common cause of death and cancer among women. Moreover, cancer of cervix is an important cause of morbimortality in the HIV-1 population being one of the most frequent cause of death. The incidence of this type of cancer has increased 2–3 more time regarding the general population. Based on the severity of the disease, treatment aspects need to be explored more in order to overcome the limitations acquired by the conventional treatment.

Nanoparticles per se or mediated drug delivery systems have been explored to target and treat cervical cancer. In addition, mucosal tissue is a critical barrier that defends the body from pathogenic infection, and typically lines the surface of internal organs and body cavities. Within the female reproductive tract (FRT), vaginal mucosa is composed of three layers that possess unique characteristics relevant to their protective functions. The secreted mucus layer, located on the apical side of the mucosa, is made of polymerized mucin fibers and globular secretions. Human cervicovaginal mucus (CVM) is a viscoelastic gel containing a complex mixture of mucins, shed epithelial cells, microbes and macromolecules, such as antibodies, that together serve as the first line of defence against invading pathogens. Cervical mucus is produced by the ectocervix and forms CVM that locally lines the cervix, as well as the vaginal compartment. Mucin, a major structural component of mucus, is a high-molecular-weight anionic molecule that non-covalently interacts to create a mesh-like framework. Depending on arrangement, size, and porosity of these mucin fibers, active agent and dendrimer/nanoparticle penetration are impeded, similar to that of virus penetration. Despite these drawbacks, using human CVM is a relatively straightforward technique that allows for rapid data interpretation, which can be valuable to the design of dendrimer/nanoparticle formulations.

Previously, the histopathological examination did not show vaginal irritation, inflammation, lesions or damage in vaginal mucosa after administration of G2-S16 at different concentrations and times in female mice and rabbits. This dendrimer/nanoparticle did not modify vaginal microbiota neither *in vitro* nor *in vivo*. New nanoparticles will be assayed with the objective to obtain muco-adhesion (for retention) or mucus penetration (for distribution). Based on severity of the disease, treatment aspects need to be explored more in order to overcome the limitations acquired by conventional treatment. We will study different nanoparticles for the effective treatment of cervical mucus in human cervical cancer non-infected or HIV infected.

Bola Dendrimers with Short Spacer Carrying Antioxidant Moiety

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Oxidation is a process that occurs naturally in the body when oxygen combines with reduced molecules, such as carbohydrates or fats, and provides energy [1]. Oxidative metabolism is essential for survival of cells. A side effect of this dependence is production of free radicals and other reactive oxygen species that cause oxidative damage. Defence mechanisms against the effects of excessive oxidations are provided by action of various antioxidants and the need to measure antioxidant activity is well documented [2].

Here we present a new class of dimeric peptide dendrimers built from two different branched fragments connected with short spacer (bola-type structure) carrying moieties with antioxidant and radical quenching properties – *p*-aminobenzoic acid (PABA) and *p*-aminosulfonic acid (PAS).

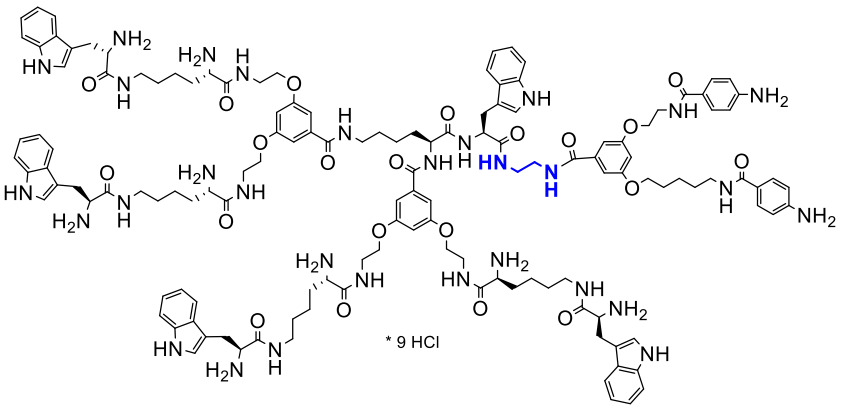

Figure. Example of dendrimer with PABA moieties

Dendrimers were tested for antioxidant capacity (FRAP – Ferric Reducing Antioxidant Power) and radical quenching ability (DPPH test). Antioxidant activity observed for dendrimers containing PABA residue was higher than for monomeric structures studied before [3].

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Amine Partially Functionalized Polyester Dendrimers for Doxorubicin Delivery

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Doxorubicin (DOX) is one of the most used anticancer drugs despite being associated with highly severe side effects, like life-threatening cardiotoxicity [1]. Polyester dendrimers based on monomer 2.2-bis(hydroxymethyl)propionic acid (bis-MPA) are very promising systems for biomedical applications due to their biodegradability properties [2]. Here, bis-MPA-based dendrimers were evaluated as carriers for delivery of DOX in cancer cells. Generation 4 and 5 bis-MPA-based dendrimers with hydroxyl termini were used (B-G4-OH, B-G5-OH), together with dendrimers partially functionalized with amine groups at the periphery (B-G4-NH2, B-G5-NH2). The goal was to compare the effect of different functional groups on dendrimer behaviour without compromising too much their cytotoxicity as, as it is well-known, dendrimers possessing a high positive charge may be quite cell damaging. Generations 4 and 5 poly(amidoamine) (PAMAM) dendrimers were also tested for comparison.

Results showed that bis-MPA-based dendrimers without DOX were cytocompatible, independently of the exposed surface groups. Both B-G4-NH2 and B-G5-NH2 dendrimers retained a higher number of DOX molecules during the loading process compared with the B-G4-OH and B-G5-OH ones. However, in *in vitro* drug release studies, drug release was faster in these systems too. These results were consistent with cytotoxicity assays performed in several cancer cell lines (A2780, CAL-72, and MCF-7 cells) by analysis of the dose-response profile. Conversely, the B-G4-OH and B-G5-OH dendrimers had a lower loading capacity but were able of delivering DOX in a sustained manner, thus showing a lower cytotoxicity for similar periods of time.

In conclusion, results revealed that the drug delivery behaviour of bis-MPA-based dendrimers was dependent on surface functionalization and that hydroxyl-terminated dendrimers are better candidates for DOX delivery.

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Nanoparticle-in-Microparticle Oral Delivery System of β-Casein Micelles for Co-Administration of Antiretrovirals

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Combination chemotherapies have been a mainstay in treatment of disseminated malignancies such as tuberculosis (TB) and human immunodeficiency virus (HIV) infection for decades and are becoming increasingly more clinically relevant in other diseases (e.g., cancer) to increase efficacy and overcome multidrug-resistance. However, production of fixed-dose combinations (FDC) remains a challenge, especially when they are based on nanotechnologies where more than one drug has to be co-encapsulated in the same carrier at optimal therapeutic ratio.

Aiming to explore novel nanotechnology strategies for the co-encapsulation of double and triple drugs combinations, in this work, we report for the first time on the encapsulation of two antiretroviral (ARV) combinations within the core of β-casein (bCN) micelles: (i) tipranavir (TPV):efavirenz (EFV) in a bCN to drugs mole ratios of 1:8:8 (bCN:TRP:EFV) and (ii) darunavir (DRV):ritonavir (RIT):efavirenz (EFV) in a bCN to drugs mole ratios of 1:8:6:1 (bCN:DRV:EFV:RIT). Encapsulation efficiency is approaching 100 %. The drugs-free and drugs-loaded bCN formulations were characterized by Dynamic light scattering (DLS) and by Cryo-Transmission Electron microscopy (Cryo-TEM), both showing increase in micelles size from 26.0 nm ± 2.0 nm to 37.0 nm ± 2.5 nm upon drugs encapsulation. Wide angle X-ray diffraction (WAXD) further shows that the encapsulated drugs are amorphous. In addition, our drugs-loaded bCN dispersions can be freeze-dried, without the use of any cryoprotectant, to ensure long-term physicochemical stability and re-dispersed in phosphate buffer saline (PBS) without any detrimental change in their properties. Then, to ensure chemical stability and prevent drugs release in the stomach, we re-encapsulated the drugs-loaded bCN micelles within the pH sensitive Eudragit® L100 copolymer using the Nano Spray Dryer B-90 HP to make protective microparticles.

Overall, newly constructed drugs loaded bCN microparticles helpful to enhance drugs bioavailability due to localized release of drugs in small intestine and can be useful in oral delivery of hydrophobic drug combinations.

Epithelial Transcytosis for Oral Delivery of Macromolecules and Nanomedicines

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Oral administration is the ultimate drug delivery route due to patient acceptability. However, this is currently not viable for complex therapeutics, such as biologics or nanomedicines, which suffer from very poor (usually clinically negligible) penetration across the intestinal barrier. Current approaches to improve oral delivery typically employ 'absorption enhancers' that non-selectively disrupt and increase the intestinal permeability. However, safety concerns, e.g. associated with many surfactants, have hindered the clinical translation of this century-old approach.

Central to safe and effective oral delivery of biologics and nanomedicines is selective intestinal translocation of the drug/drug delivery system without disrupting this physiologically-important barrier. This has led to fabrication of drug systems that explore biological transport processes to shuttle therapeutics across the intestinal barrier. Various biological transport pathways have been explored. We will share our experience and observations related to the use of epithelial transcytosis (for neurotensin, albumin and transferrin) for intestinal delivery of macromolecules and nanoparticles.

Fabrication of systems was achieved in different ways. Peptide-protein constructs were created by molecular biology approaches; polystyrene model nanoparticles were surface modified with proteins transport ligands via physical adsorption and human serum albumin nanoparticles were formulated via desolvation. Cell uptake and transport utilised the intestinal Caco-2 model (polarised cell monolayers) and was assessed by fluorescence (confocal microscopy imaging and quantitation by fluorimetry).

We will summarise our recent work, both published [1–3] and unpublished, on intestinal delivery of macromolecules and nanoparticles. Where possible, comparison of the potential of different biological transport systems with respect to material uptake and transepithelial transport will be made.

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Precision Chemotherapy with a Photoswitchable Drug

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Chemotherapy is one of the most common treatments for cancer. However, its therapeutic potential is in many cases seriously unsatisfactory due to the nonspecific drug distribution and off-target effects. Innovative chemotherapeutic treatments based on nanomedicine can increase therapeutic efficiency while reducing side effects to normal tissues. One emerging approach is photopharmacology, which relies on light-controlled nanoscale changes in structure of a drug to turn its pharmacological activity "on" and "off" on demand.

We have designed and developed the first photoswitchable inhibitor of the human dihydrofolate reductase and named it Phototrexate. Preliminary studies in vitro and in zebrafish demonstrated that Phototrexate behaves as a potent antifolate after illumination with UVA light and that it is nearly inactive in its dark-relaxed form [1, 2]. Moreover, early safety screenings revealed that it can be considered reasonably safe for further studies in animal models of disease.

Overall, Phototrexate is emerging as a new drug candidate towards the development of high-precision photochemotherapy.

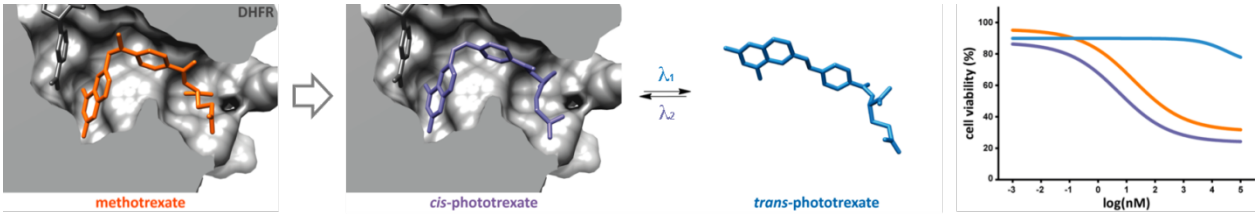

Figure 1. Phototrexate, in its *cis* configuration (in violet), can mimic the bound conformation and effects of Methotrexate (in orange).

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Surfactant-Assisted Microwave Processed ZnO Nanoparticles with Optimized Surface-to-Bulk Defect Ratio For Potential Biomedical Application

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Owing to a wide band gap energy (3.37 eV at RT) and a large exciton binding energy (60 meV), ZnO nanoparticles (ZnONP) have a diverse application, e.g. in electronics, optoelectronics, photocatalysis. Besides, ZnONP have a great potential in medicine for bioimaging, drug/gene delivery or as antimicrobial and anticancer agents. One of suggested governing mechanisms of the mentioned biological activities of ZnONP is based on formation of reactive oxygen species (ROS). When ZnONP absorb photon with energy equal or greater than its band gap, electrons are excited from the valence band (VB) to the conduction band (CB) leaving the holes in VB. Furthermore, the photogenerated holes (h^+) and electrons (e^-) migrate from bulk to surface. The photogenerated holes at the VB react with water molecules adsorbed at the particle surface to produce hydroxyl radical, while electrons in CB react with oxygen molecules generating anionic superoxide radical $O_2^{-\bullet}$. Superoxide radicals can be transformed in highly reactive $OH^\bullet$ and so on [1]. Derivatives of this active oxygen can damage the bacterial/tumor cells [2]. However, in sufficiency ROS can damage normal cells as well. Thus, an understanding of ZnONP crystal structure-activity relationship and mechanism of ZnONP-related products formation and their consequent activity is crucial for the design of safe ZnONP based biomaterial for application in treating diseases like cancer.

A series of ZnONP samples were synthesized by microwave processing of precipitate in the presence of a small amount (5 wt.%) of surfactants CTAB and citric acid. The particles crystallinity and purity were investigated by X-ray diffraction, Raman and ATR-FTIR spectroscopy. The particles morphology and texture properties were observed with field emission scanning electron microscopy (FE–SEM) and nitrogen adsorption–desorption isotherm, respectively. The optical properties were studied using UV–Vis diffuse reflectance and photoluminescence (PL) spectroscopy. ZnONP samples with different surface-to-bulk defect ratio were examined on ROS formation and antimicrobial activity. Future studies will be conducted with an aim to correlate surface-to-bulk defect ratio in ZnONP with mechanism of ROS formation and their cytotoxicity to normal and cancerous cells.

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Graphene Oxide-Based Nanocomposites Decorated with Zink Oxide Nanoparticles as Antibacterial Agent

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Development of antibiotics has played a significant role in controlling a number of bacterial infections. However, improper use and overuse of antibiotics have led to the development of multidrug resistance in many bacterial species.

One of the most promising methods against drug-resistant bacteria can be surface-modified materials with biocidal nanoparticles and nanocomposites. Herein, we present a nanocomposite with zink oxide nanoparticles (ZnO-NPs) on the surface of graphene oxide (GO) as a novel multifunctional antibacterial. Toxicity on gram-negative bacteria (Escherichia coli), gram-positive bacteria (Staphylococcus aureus) was evaluated by analysis of cell morphology, ultrastructure, assessment of cell viability using the XTT assay, analysis of cell membrane integrity using the lactate dehydrogenase assay, and reactive oxygen species production. Presented nanocomposite shows much higher antimicrobial efficiency toward both type of bacteria. After co-incubation the bacterial cells for 24 h with ZnO-GO nanocomposite we observed inhibition of the growth of all tested microorganisms with varying degrees. A disruption of membrane functionality from an interaction between released nanoparticles/ions and the cell membrane and extensive cell membrane damage caused by the formation of ROS ultimately caused damage to the cell due to oxidative stress. This action is most probably due to an increase in cell membrane and wall penetration by nanoparticles.

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Preparation and Optimization of Novel Nanoemulsions for Oral Drug Delivery

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Currently nanoemulsions (NE) represent promising formulations for increasing oral bioavailability of poorly water-soluble drugs [1]. Their main advantages rely on high stability and capability to enhance solubility of lipophilic compounds, easy manufacture and scale-up [2, 3].

In this work, the design and development of a novel versatile NE by a modified emulsion phase inversion (EPI) technique is presented. The system made of FDA approved excipients was composed of a medium chain triglycerides (MCT) oil core stabilized by a mixture of hydrophilic (Polyoxyethylene (40) stearate) and hydrophobic (oleoyl polyoxyl-6 glycerides) surfactants. To identify the region of interest of suitable formulations, an experimental design using a ternary phase diagram was developed. The optimisation of physicochemical parameters such as surfactant to oil ratio (SOR = 2.9) and surfactant composition (SMR = 2.5) led to the obtainment of stable NE with a hydrodynamic size of around 100 nm and a slightly negative surface charge (-9.1 ± 1 mV). The robustness and transposability of the system were evaluated and the optimized NE was scaled up 10-fold. NE efficiently encapsulated the hydrophobic model drug tacrolimus (encapsulation efficiency around 100 %). Following *in vitro* studies in GI media, we showed that once encapsulated in the NE tacrolimus was released in a sustained manner (50 % in simulated gastric fluids (SGF) and 40 % in fasted state simulated intestinal fluids (FaSSIF-V2) up to 2 hours). To increase the shelf-life of the finished product by preserving it in a more stable dry state, NE were converted in powder by the freeze-drying technique. After reconstitution in water no variation of NE physicochemical properties was observed [4]. Then, to assess the structural properties of the NE, morphological analysis using differential scanning calorimetry (DSC) and X-ray diffraction (XRPD) was performed.

Results proved that NE shell was amorphous when in colloidal suspension, while upon drying it became crystalline. Overall, we demonstrated that through accurate choice of excipients and thorough control of formulation parameters, final properties of NE can be finely tuned, unveiling new possibilities for exploitation of this system for oral drug delivery.

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Glycoconjugate Architecture Steers Affinity to Galectins

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LacNAc (Galβ4GlcNAc) is a typical carbohydrate ligand of galectins – lectins regulating, i.a., intercellular communication, adhesion and signaling [1]. Human galectins participate in a number of pathologies including cancerogenesis, metastatic formation, inflammation or fibrosis. They are prospective targets for therapeutical applications where selectivity for one particular galectin is highly desirable.

In the present study, we synthesized *N*-(2-hydroxypropyl)methacrylamide (HPMA) copolymers decorated with LacNAc epitope in various concentrations and architectures. Functionalized LacNAc was prepared using □-galactosidase from *Bacillus circulans* [2]. In a structure-affinity relationship study we compare the difference in affinity between LacNAc distributed statistically on the polymer backbone or nested on bi- and trivalent phenyl branches. The affinity of prepared glycopolymers to galectins was determined in ELISA-type assay. The manner of the LacNAc presentation on the HPMA copolymer brings a clear discrimination between galectins, reaching avidity in nanomolar range. The prepared glycopolymers are attractive for *in vivo* use due to good water solubility, no toxicity and immunogenicity [3].

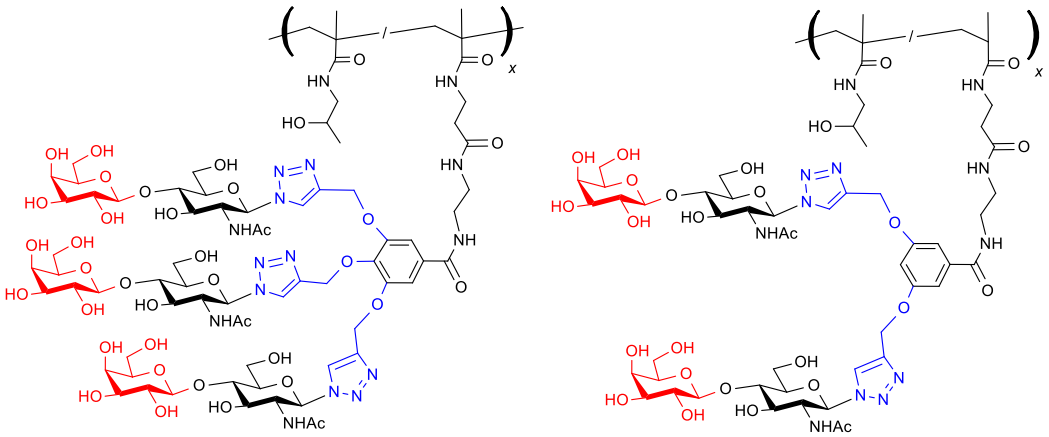

Figure 1. Structures of di- and tribranched LacNAc-decorated HPMA copolymers.

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Biological Properties of Cationic Dendritic Systems

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At present, dendrimers exhibit a strong impact on the field of biomedicine and pharmaceutical industry, due to their multivalency, monodispersity, flexibility as well as their well-defined size and structure [1]. Cationic dendritic macromolecules present significant antibacterial activity, as a consequence of their amphiphilic behavior [2].

Here, we have synthesized different cationic, homo- and heterofunctionalized dendritic systems, employing thiol-eno click chemistry and different strategies for quaternization [3]. Their antibacterial, haemolytic and toxicity properties have been evaluated, results being dependent on type of dendritic core, cationic group, presence of PEG, type of focal point in dendrons. Finally, interaction of selected dednrimers with biofilm was also analysed.

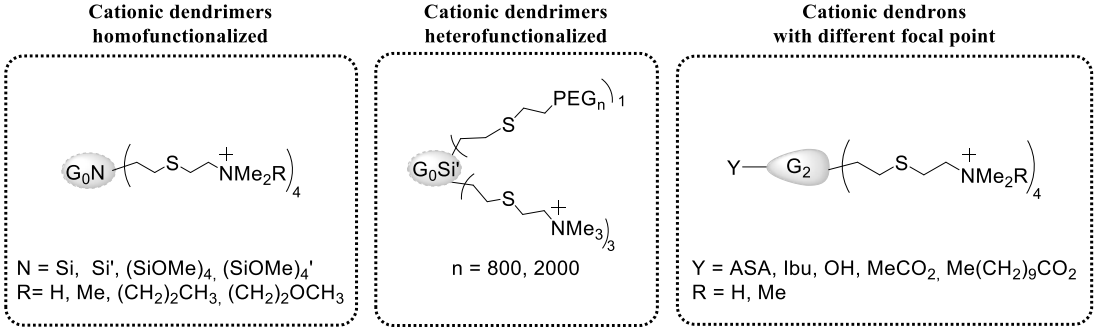

Figure. Type of dendritic systems studied.

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Biomimetic Impedance-Based Assessment of Biological Effects of Anticancer Nanoparticles

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Innovative realistic biomimetic instruments and methods that can assess effects of anticancer nanoparticles in an *in vivo* like, label-free, less prone to interferences, robust and cost-efficient manner are essential for ensuring correct hazard and risk/benefit evaluations. Such devices and assays can shorten the time needed for novel nanotheranostics to reach the market and clinical applicability by reducing the needs for in vivo animal testing. They can also contribute to replacing animal use, in conformity with the 3 R principles.

Traditional assays employ reagents and markers that can distort cellular responses and can be prone to nanoparticle-induced interferences, thus producing unreliable results. Conventional *in vitro* tests are usually performed under static, non-physiological conditions and thus do not reflect the *in vivo* exposure situation. Moreover, nanoparticle-agglomerates sediment first and the cells come in contact mostly with these. Label-free methods are less prone to nanoparticle-induced interferences and can therefore provide more reliable results.

Here we present a multi-channel microfluidic device with integrated microelectrode arrays, which can provide a well-controlled perfusion of nanoparticle dispersions under physiologically relevant flow conditions. The system allows both impedance- and microscopy-based real-time monitoring of cells and biomimetic structures.

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Diamond Nanoparticles Reduce Proliferation of Glioblastoma Multiforme through G0/G1-Phase Arrest

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Glioblastoma multiforme (GBM) is a type of malignant primary brain tumor. The presence of necrotic areas, atypical vascularization, and atypical cells with nuclear pleomorphism and high proliferative ratio are this cancer's most important features. Despite many years of research, glioma is still the deadliest form of human cancer. It is, therefore, necessary to seek innovative experimental therapies to treat this type of tumor.

Diamond nanoparticles (ND) exhibited antiangiogenic and proapoptotic properties *in vitro* in glioblastoma multiforme (GBM) cells and in tumors *in vivo* [1]. Moreover, ND inhibited adhesion leads to suppression of migration and invasiveness of GBM [2]. It has been hypothesized that ND might also inhibit proliferation and cell cycle [3]. Experiments were performed *in vitro* with GBM eU87 and U118 cells after 24 h and 72 h of treatment. Analysis included cell morphology, viability, apoptosis, and cell cycle analysis, double timing assay, and gene expression. The results confirmed the antiapoptotic effect of ND on U87 and U118 cells. After 72 h of ND treatment, the expression levels of Rb, CycD, and CycE in the U118 cells, and E2F1, CycD and CycE in the U87 cells were significantly lower in comparison to those in the control group. The decreased expression of these cyclins inhibited the S phase transition.

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Physico-Chemical and Preclinical Characterization of Polymeric Nanoparticles for Therapy and Diagnosis

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Polymeric nanoparticles offer a great flexibility adapting its chemistry composition, size, stability, morphology and surface functionality [1]. As a result, they are used in Biomedicine as transporters of drugs and diagnostic agents for a wide range of applications in diagnosis, therapy and theranostics [2]. We are focussed on the design, synthesis, scale-up, PCC and preclinical characterisation of nanoparticles to be applied in biomedicine.

Herein we will show some techniques and protocols that we are applying to characterise nanoparticles of different nature and different sizes. Several parameters are measured such as size and aggregation (using DLS, confocal microscopy, SEM, TEM and AFM), surface (zeta potential, EDX), composition (EDX, mass cytometry, FLIM, flow cytometry), drug release by LC-MS/MS, stability and batch to batch consistency, together with preclinical characterization (toxicity, in vitro immunology/hematology, etc) [3].

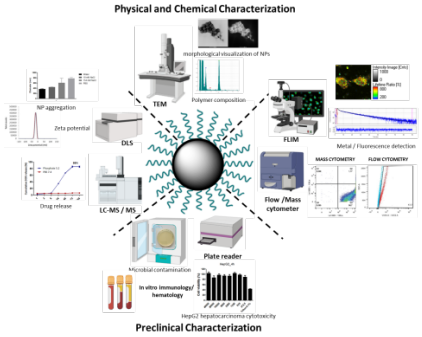

Figure. Physicochemical and preclincial characterisation techniques applied in NanoChemBio lab.

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