COST ACTION 17140

Working Group 2

ONLINE CONFERENCE

"Characterisation of nanomaterials towards safe and efficient nanodrugs"

BOOK OF ABSTRACTS

June 22–23, 2021

Zagreb, Croatia
Toulouse, France
Lodz, Poland

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Instituto de Nanociencia y Materiales de Aragón, CSIC-Universidad de Zaragoza & CIBER-BBN, Spain

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14:30 – 15:30 ***Poster viewing and General discussion on topics***:
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2 - Quality control of nanopharmaceuticals and nano-drug delivery systems throughout the production

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Day 2 (23rd June 2021)*

9:30 – 9:40 ***Openning and introduction***

9:40 – 10:40 ***Plenary lecture***
Dietmar APPELHANS "Analytical tools for the characterization of (surface-active) protein therapeutics and cell biomimetics"
Leibniz-Institut für Polymerforschung Dresden e.V., Dresden, Germany

10:40 – 11:00 Coffee break

11:00 – 11:15 ***Short oral***
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11:15 – 11:30 ***Short oral***
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11:30 – 11:45 ***Short oral***
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11:45 – 12:00 ***Short oral***
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12:15 – 12:30 ***Short oral***
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12:30 – 13:30 Lunch break

13:30 – 13:45 ***Short oral***
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13:45 – 14:00 ***Short oral***
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14:00 – 14:15 ***Short oral***
Martins Cláudia, Barbosa Catarina, Araújo Marco, Oliveira Maria, Aylott Jonathan W., Sarmento Bruno "Unravelling the chemotherapeutic and immunomodulatory effect of glioblastoma-targeted nanoparticles through a novel tumor niche-recapitulating 3D spheroid construct"

14:15 – 14:30 Coffee break

14:30 – 15:45 ***Poster viewing and general discussion on topics***:
3 – Nano-bio interactions governing the efficacy of nanodrugs
4 – Techniques and methods for evaluation of drug loading and release from nanoformulations

15:45 – 16:00 ***Closing***

**All events are schedulded according to the Central European Time (CET)*

PLENARY LECTURES

Analytical tools for the characterization of (surface-active) protein therapeutics and cell biomimetics

Dietmar APPELHANS[1], E. Geervliet[2], L. Baiamonte[1], S. Moreno[1], S. Boye[1], A. Lederer[1], R. Bansal[2]

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Background: The development of extracellular matrix protein therapeutics, based on polymersomes, for disease treatment (e.g. liver fibrosis, wound treatment and matrix of cancer) requires stringent protocols for their synthesis and characterization to build the platform for any medical applications. The key features for their successful application are very diverse as known from other drug nanoformulations. Besides showing synthetic approaches for protein therapeutics, main concerns are the use of different characterization techniques to determine the accessibility, (colloidal) stability, (long-term) activity and biological effect of protein therapeutics. Here, it will be shown results of matrix metalloproteinase-1-(MMP-1)-post loaded polymersomes (MMPsomes) for alleviating liver fibrosis.[1]

Experimental: MMPsomes have been characterized by different techniques such as DLS, cryo-TEM, zetapotential and asymmetrical flow-field flow fractionation combined with light scattering techniques (MALS and DLS), including enzyme and biological assays.[1]

Results: Enzymatically active MMPsomes with defined surface location are storage-stable fabricated by a post-loading of Psomes with MMP-1. Synthetic approach of MMPsomes can be transferred into a biologically applicable approach. MMPsomes outline the requested biological effects: dose-dependent effects of MMP-1, and effects of MMPsomes *versus* MMP-1, empty polymersomes (Psomes) and MMP-1 + Psomes on gene and protein expression of collagen-I, MMP-1/TIMP-1 ratio, migration and cell viability towards TGFβ-activated human HSCs. Positive therapeutic effects of

MMPsomes, compared to MMP-1, are given by carbon-tetrachloride (CCl4)-induced early liver fibrosis mouse model. MMPsomes also inhibit intra-hepatic collagen-I (ECM marker, indicating early liver fibrosis) and F4/80 (marker for macrophages, indicating liver inflammation) expression.

Conclusion: MMPsomes are surface-active vesicle-based ECM therapeutics for the treatment of early liver fibrosis. The shown principles for protein-post loaded polymersomes[1,2] are also applicable to other proteins (e.g. hyaluronidase) and nanoparticles for tailoring the environment of affected cells and tissues.

References:

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2. Gumz, H.; Boye, S.; Iyisan, B.; Krönert, V.; Formanek, P.; Voit, B.; Lederer, A.; Appelhans, D. *Advanced Science* **2019**, *6*, 1801299.

Acknowledgments: D. Appelhans thanks all other non-named contributors to this research topic.

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Dr. Appelhans´ group carries out the design, synthesis and deep physical-chemical characterization of multifunctional, bioactive and responsive polymer structures and associates for use in nanomedicine. This work comprises especially dendritic polymers with special emphasize on glycodendrimers, but also responsive polymersomes and nanocapsules as well as multicompartment structures for drug delivery as well as cell mimics.

Hybrid Nanoparticles for Therapy and Diagnosis: Au NanoPrisms for Gastrointestinal Cancer

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In the last decades, inorganic nanoparticles have been steadily gaining more attention from scientists from a wide variety of fields such as material science, engineering, physics, or chemistry. The very different properties compared to that of the respective bulk, and thus intriguing characteristics of materials in the nanometre scale, have driven nanoscience to be the centre of many basic and applied research topics. Moreover, a wide variety of recently developed methodologies for their surface functionalization provide these materials with very specific properties such as drug delivery and circulating cancer biomarkers detection. In this talk we describe the synthesis and functionalization of magnetic and gold nanoparticles as therapeutic and diagnosis tools against cancer.

Gold nanoprisms (NPRs) have been functionalized with PEG, glucose, cell penetrating peptides, antibodies and/or fluorescent dyes, aiming to enhance NPRs stability, cellular uptake, and imaging capabilities, respectively. Cellular uptake and impact were assayed by a multiparametric investigation on the impact of surface modified NPRs on mice and human primary and transform cell lines. Under NIR illumination, these nanoprobes can cause apoptosis. Moreover, these nanoparticles have also been used for optoacoustic imaging, as well as for tumoral marker detection using a novel type of thermal ELISA and LFIA nanobiosensor using a thermosensitive support.

Jesús M de la Fuente

Prof. Jesús Martínez de la Fuente (Barakaldo, 1975) created his own research group (BIONANOSURF Group) at the Univ of Zaragoza in 2007, becoming internationally recognized in nanomaterials and biofunctionalization. The multidisciplinary nature of the group facilitates research and development in numerous areas, including biosensors, gene therapy, magnetism, photochemistry, surface chemistry and molecular metal oxides, among others. He has extensive experience in the synthesis and characterization of novel nanomaterials and their biofunctionalization for the use and development of the next generation of nanobiosensors and nanotherapeutics. In 2009, he founded the spin-off Nanoimmunotech SL. He has also been a pioneer in the application of gold nanoparticles in gene therapy and he has developed a methodology for the use of gold nanoparticles functionalized with carbohydrates (glyconanoparticles) for the study of biological processes (embryogenesis, cancer, inflammation, etc.). He has been PI of research projects with a total budget of more than 6 M€. 75% of this budget is derived from European projects (1 ERANET (Coord); 1 ERC-StG (Coord), 1 ERC-POC (Coord), 7 MSCA-IOF/IEF/IF (Coord), 1 ENMII, 1 TRANSCANII (Coord), 1 FP7, 3 H2020-NMBP); 10% comes from collaborations with companies (CASEN-FLEET, ORPHAN DRUG-RECORDATI, MECWINS, NB, NIT, PROTEOMIKA, BSH, VIRBAC); and the remaining 15% comes from research projects of national calls. In 2010 he was awarded the Aragón Investiga prize "Young Researchers". In 2013 he was awarded by the Shanghai Administration with the 1000 Talent Plan program to be a visiting professor at the Jiao Tong University of Shanghai. Since 2014, he is a permanent researcher at the Instituto de Nanociencia y Materiales de Aragon-CSIC.

ORAL PRESENTATIONS

Inflammatory potential of magnetite nanoparticles is determined by coating in murine renal podocytes

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Drug-induced nephrotoxicity is important dose-limiting factor and a major reason for late-stage failures of novel drugs in clinical trials [1]. Therefore, early prediction of nephrotoxicity in preclinical studies is of high importance. On this background, we investigated impact of magnetic nanoparticles (MNPs) on renal cells responsible for the blood filtration in the kidneys – podocytes.

Primary murine glomerular podocytes were isolated from C57BL6 kidneys and were exposed to MNPs with either polyethylene glycol (PEG) or bovine serum albumin (BSA) coating.

Both types of MNPs induced inflammatory response, however via different mechanisms. MNPs with BSA coating triggered rather early and with PEG coating rather late inflammatory response. This in case of PEG MNPs led to markedly elevated protein levels of cytokines compared to BSA coated MNPs. Interestingly, actin fiber remodeling and subsequent cell shape alteration due to MNPs exposure, which especially in case of podocytes is directly linked to loss of their function and defective filtration of the blood, was similar between the two types of MNPs.

Altogether, the results show substantial role of coating selection in nanoparticle-induced cellular toxicity. Understanding the nano-bio interactions and considering all multiple variables might help to select the safer nanotherapeutics and so facilitate translation of nanoparticle-based platforms into clinics.

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Nanoemulsions as delivery systems of nucleic acids against breast cancer – are they ready-to-use after long-term storage?

Selen Isar [1†], Hasan Akbaba [1], Yiğit Şahin [2], Meric A. Altinoz [3], Ayşe Nalbantsoy [4], Gülşah Erel-Akbaba [5], Yücel Başpınar [1 *]

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Background: Ready-to-use gene delivery systems like nanoemulsions (NEs) are requested and required, otherwise delivery systems have to be prepared freshly each time before application.

There are several NEs available in the pharmaceutical market like Diazepam® Lipuro, Disoprivan®, Diprivan®, Etomidat® Lipuro, Intralipid®, Lipofundin®, Propofol 1%/2% Fresenius and Stesolid®. Diprivan gained notoriety by the death of Michael Jackson in 2009. Another famous compound is erucic acid (EA), a component of Lorenzo's oil, which was used by the parents of Lorenzo Odone, an adrenoleukodystrophy (ALD) patient, till his death in 2008. Several studies have reported that EA may act anti-tumoral on C6 glioma, melanoma, neuroblastoma, and glioblastoma.

This study is focused on the preparation and characterization of NEs as a model for the delivery of nucleic acids against breast cancer cells MDA-MB-231 and MCF-7. Some advantages of NEs are

their versatile routes of application like oral and parenteral, and the loading of hydrophilic and lipophilic drugs.

Due to the fact that nucleic acids are anionic, they should be loaded to cationic delivery systems by electrostatic interaction. For preparing cationic (C) NEs, the cationic compounds phytosphingosine (PS) and didodecyldimethylammonium bromide (DDAB) were used. It was reported that PS may act anti-apoptotic.

Experimental: Five different CNEs (CNE 1, 2, 3, 4 and 5) and one NE were prepared with microfluidization method by investigating the microfluidization duration of 1, 2, 3, 4, 5, 6, 8 and 10 minutes, and with a pressure of 600 bar. As oil components octyldodecanol (OD) and lauroglycol 90 (LG), with concentrations of 5, 10 and 20 %, as cationic agents PS, PSHCl and DDAB and EA as co-lipid were used. The prepared CNE s and NE were characterized in terms of droplet size (DS), polydispersity index (PDI), and zeta potential (ZP), long-term stability after storage at 25 and 40 °C, complexation with pDNA, release and cytotoxicity on breast cancer cells MDA-MB-231 and MCF-7 cells.

Results: All prepared CNEs showed appropriate properties like a small DS of <200 nm, a narrow size distribution, expressed as PDI <0.3, and a high ZP of >+30 mV. Long-term stability studies of 3 months at 25 and 40 °C have shown that CNE 1, 2 and 3 are stable, but CNE 4 and 5 revealed phase separation after storage of six months at 40 °C. All CNEs formed complexes with pDNA, except CNE 5. The cytotoxicity studies on breast cancer cells revealed that the viability of MDA-MB-231 cells was reduced to 20% by CNE 1, to 11% by CNE 2, to only 80 % by CNE 3, to 10 % by CNE 4 and to 40 % by CNE 5. Viability results using MCF-7 showed a decrease to 13 % by CNE 1, 2 and 3, to only 80 % by CNE 3 and to 54 % by CNE 5. All CNEs forming complexes with pDNA showed appropriate release properties. The highest number of transfected cells were obtained after the use of CNE 2 containing PS and EA.

Conclusion: Giving a resume about the preparation of CNEs and their characterization in terms of DS, PDI, ZP, long-term stability, complexation with pDNA, cytotoxicity and transfection, it can be stated that CNE 2, containing PS and EA as essential compounds, is appropriate for the purpose of delivering pDNA.

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Acknowledgments: This is dedicated to the memory of Selen Isar, a graduate student, who lost her fight against stroke after 135 days. She was an outstanding student and loved by her family and colleagues.

Antibacterial and antiviral properties of metal nanoparticle-based materials

Anna-Liisa Kubo[1], Kai Rausalu[2], Natalja Savest[3], Grigory Vasiliev[1], Eva Zusinaite[2], Mihkel Viirsalu[3], Andres Krumme[3], Andres Merits[2], Olesja Bondarenko[1]

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Background: Development and characterization of new antibacterial and antiviral materials is crucial in the context of the on-going SARS-CoV-2 pandemic and beyond, to fight hospital-associated bacteria and viruses.

Experimental: In this study we tested antibacterial and antiviral properties of a range of metal-based nanoparticles (NPs). The efficiency of ZnO, CuO and Ag and respective metal salts was tested against influenza A virus and bacteria *Escherichia coli* and *Staphylococcus aureus*. The most effective NPs and salts were selected for the incorporation into polymers to produce antiviral and antibacterial filter materials for the face masks using electrospinning.

Results: NPs were thoroughly characterized in powders, in test environment and electrospinning solutions. Among tested compounds, CuO and $CuSO_4$-based materials demonstrated the highest efficiency against influenza A virus and were selected for the production of filter materials. Developed CuO-based filter materials efficiently inactivated bacteria *Escherichia coli* and *Staphylococcus aureus*. In the next step, the efficiency of these filter materials to inactivate SARS-CoV-2 will be tested.

Conclusion: Metal nanoparticles and respective metal salts are potent antibacterial and antiviral compounds that can be successfully incorporated into filter materials of the face masks.

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Self-assembling light-activated anticancer drugs

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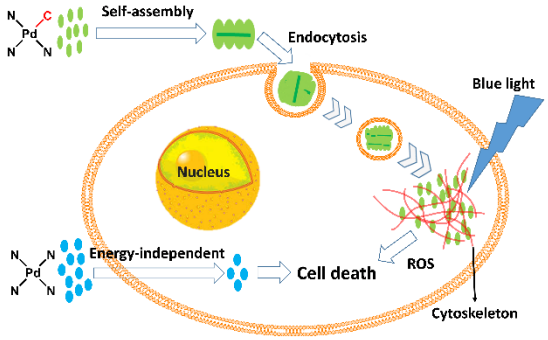
Enhanced passive diffusion is usually considered as the primary cause for the enhanced cellular uptake of cyclometalated drugs, because cyclometalation lowers the charge of a metal complex and increases its lipophilicity. In this presentation we will discuss cyclometalated palladium complexes that to self-assemble, in aqueous solutions, into soluble supramolecular nanorods. These nanorods form via the metallophilic Pd…Pd interaction, and they are stabilized in cell medium by serum proteins, in absence of which the nanorods precipitate. In cell cultures these protein-stabilized self-assembled nanorods were responsible for the improved cellular uptake of the cyclometalated compounds, which took place via endocytosis, i.e. an active uptake pathway. In addition to triggering self-assembly, cyclometalation also led to dramatically enhanced photodynamic properties. These combined penetration and photodynamic properties were observed in multicellular tumor spheroids and in a mice tumor xenograft, demonstrating that protein-stabilized nanoaggregation of cyclometalated drugs allows efficient cellular uptake also in 3D tumor models.

Overall, serum proteins appear as a major element of drug design, as they strongly influence the size and bioavailability of supramolecular drug aggregates, and hence their efficacy in vitro and in vivo.



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A high-throughput method for drug release measurements in complex media

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Background: Bringing a promising nanomedical candidate towards the clinic requires evaluation of product quality and performance. This can be verified through a cascade of *in vitro* and *in vivo* experiments. Of these, drug release from the nanoformulation provides essential information about the pharmacokinetics and hence the bioavailability of the drug. Even though *in vitro* drug release is a widely studied parameter in characterization of nanomedicines, no standardized methods are available today. Most often, *in vitro* drug release experiments are performed at 37 °C in a buffered salt solution with pH 7.4 to simulate physiological conditions. Such systems are, however, not able to truly mimic the *in vivo* environment, and will therefore not accurately predict actual nanomedicine stability *in vivo*. Performing the studies in biological matrix (full blood or blood plasma) is therefore preferable, but adds complexity in the separation step, and necessitates assessment of the interactions between released drug and plasma proteins. We have implemented a robotic high-throughput drug release method, based on the novel methodology presented by Skoczen et. al in 2015. This method compensates for drug-protein interactions, and hence gives a reliable measure of the portion of available drug *in vivo [1]*.

Experimental: The nanomedicine system is incubated in media, e.g. human blood plasma, and subsequently, a stable isotope analogue of the drug is added at known concentrations, and aliquots containing intact nanoparticles, released drug and added drug analogue are separated in centrifugal filters. The filtrate and an unfiltered parallel is then extracted by an organic solvent and analysed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) to quantitate the amount of both the drug and the isotopically labelled drug. These steps are performed in a well-plate format, and all liquid handling is done by automated robotic systems. Since the isotopically labelled drug equilibrates with protein and formulation components identically to the unlabelled drug released from the nanomedicine formulation, the filtered fraction of the isotopically labelled drug gives reliable measures of the released and encapsulated drug fractions.

Results: Figure 1 illustrates drug release results obtained by applying the presented method on two different nanomedicine systems: a liposomal drug carrier (A) and a drug-polymer conjugate (B). For B, the incubation is performed in both phosphate buffered saline (PBS) and human plasma to illustrate the differences in release kinetics.

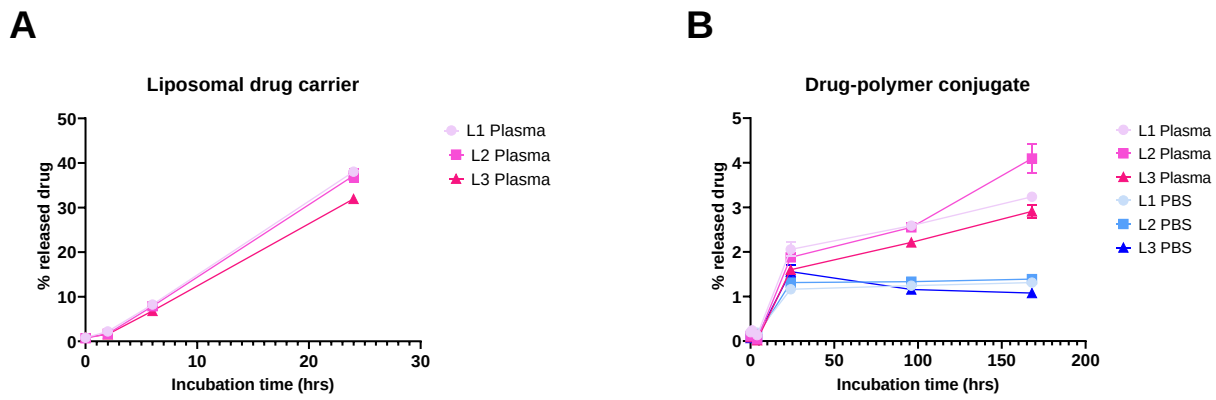

Figure 1: Example results obtained with the presented drug release methods, including a liposomal drug carrier (A) and a drug-polymer conjugate (B). L1-L3 indicates concentration levels, with L3 being the highest. All lines represent the averaged drug release calculated from 3 individual parallels.

Conclusion: The presented method gives reliable measures of free and encapsulated drug fractions, even in complex protein-rich media such as blood plasma. The high-throughput set-up simplifies simultaneous analysis of several drug concentration levels, many incubation time points and different relevant release media.

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Amphiphilic triazine-carbosilane dendrons as perspective agents for glioblastoma treatment

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Background: Glioblastoma is suppose to be one of the most aggressive and hard-to-treat type of tumor. These properties could be related on the phenotypical and morphological heterogeneity of tumor, providing the possibility to "escape" the treatment. Importantly, recent research advocates that glioblastoma progression is mostly driven by tumor cells with stem cell properties, so called glioblastoma stem-like cells (GSCs) (Kahlert et al, 2016). Targeting this subpopulation could be an effective way to treat glioblastoma, getting over the drug resistance. For this aim new chemotherapeuticals should be found. We suggest novel approach, based on using carbosilane dendrons, which proved their antitumor properties in previous experiments on other models.

Experimental: In our study we used amphiphilic triazine-carbosilane dendrones of the second (DG2) and the third (DG3) generations. For the investigation of dendrons toxicity several glioblastoma stem-like cells lines were chosen: BTSC233, JHH520 and NCH644. U87 glioblastoma cell culture in suspension state was used as a control. Cells were treated by dendrons solutions of 0,1 μM, 1 μM, 10 μM and 100 μM, then in 72 h MTT assay was performed, standard-care drug temozolomide was used as a control. For evaluation of the mechanisms providing cell toxicity Annexin V/PI apoptosis assay

was performed. Statistical análisis was done by using Mann-Whitney and Wilcoxon criteria, the difference suppose to be significant, if $p<0,05$.

Results: It was found that dendrimers have proper toxic effect on tumor cells, which is dose-dependent and generation-dependent: DG3 dendron demostrated higher toxicity than DG2. Interestingly, toxic effect of dendrons was higher than after temozolomide treatment in case of glioblastoma stem-like cells cultures (BTSC233, JHH520, NCH644), but not for U87. Treatment by dendrons but not temozolomide reached to increasing the number of late apoptotic cells in comparison with non-treated control.

Conclusion: Carbosilane dendrons demonstrated high toxic effect on the GSCs populations and they were shown to be more toxic than standard-care chemodrug. We hypothesized that dendrons could be used as an efficient platform for creating new therapeuticals to treat aggressive tumors such as glioblastoma targeting the most robust and chemoresistant subpopulation of tumor cells.

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Molecular Dynamics Approach to Rational Design of Functionalized Gold Nanoparticles for Cancer Treatment

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The current approach to nanocarrier design is mostly empirical since there is no set of "rules" which can be used as preliminary guidance. To obtain those rules, systematic investigation is needed. The experimental approach would be too expensive and time-consuming, whereas computational methods such as Molecular Dynamics (MD) provide a suitable alternative. Since gold nanoparticles (AuNPs) are increasingly used as drug carriers due to their inertness, non-toxicity and ease of functionalization, we focused on them. The long-term aim is to define the set of rules which could guide the design of new systems using MD. Working towards that goal, we are performing atomistic MD simulations of functionalized AuNPs where physico-chemical properties of the ligands are systematically varied. As a starting point, we have performed sets of simulations to study the influence of the drug's physico-chemical properties on the structure of the coating [1]. We simulated mixed-monolayer AuNPs functionalized with a zwitterionic background ligand and a ligand with a covalently bound anticancer drug (Quinolinol/Panobinostat). In simulations, the size of the gold core and composition of both ligands are kept identical with the only difference being the carried drug. With this approach we can ensure that obtained differences in the coating conformation are exclusively the result of the drug's physico-chemical properties. The ratio of the ligands was varied to investigate the effect of initial drug concentration. Results show that hydrophobicity is the dominant effect. Hydrophobic structures

tend to decrease their solvent-accessible surface area by bending towards the gold core while exposing hydrophilic parts to the solvent. Polar background ligand, although shorter than the ligand carrying the drug, tends to be more dominant on the surface in contact with water, showing that the ligand length does not play a primary role in this case. The amount of the less polar drugs on the NP-water interface increases with increasing initial drug concentration in the system, showing that the initial drug concentration has an effect. Although the investigation is ongoing, preliminary results show that it is possible to observe the trend, and with sufficient number of simulations, the generalized guidelines can be obtained.

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Enhanced oral bioavailability of anticancer drugs using supersaturable self-emulsifying drug delivery systems

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Background: To overcome limited solubility and bioavailability of drugs following oral administration, lipid-based drug delivery systems have raised considerable attention due to the ability to present the drug in a solubilised state in their lipid excipients, facilitating gastrointestinal absorption. Among them, self-microemulsifying drug delivery system (SMEDDS) offer numerous advantages, including i) thermodynamic stability ii) small droplets size which provides a large contact surface between the drug and the intestinal mucosa, maximising absorption iii) simple manufacturing process and ease of scale-up. To further improve drug stability, here we designed supersaturable (S-SMEDDS) for oral administration of a novel benzimidazole (BI) anticancer drug [1].

Experimental: Conventional SMEDDS and S-SMEDDS were optimized and physico-chemical characterized. Stability in simulated gastrointestinal fluids (SGF, pH 1.2 and SIF, pH 6.8) was also studied. The cytocompatibility of the systems and the ability to modulate the epithelial permeability were assessed *in vitro* on Caco-2 cells. *In vivo* pharmacokinetic studies were performed after oral administration to healthy mice to determine the advantage of S-SMEDDS in enhancing the systemic absorption of BI.

Results: Firstly, ternary and pseudoternary phase diagrams were constructed to generate an optimized conventional SMEDDS formulation able to form neutral microemulsions of around 20 nm *in situ* in

presence of intestinal fluids. To increase drug loading and stability, the addition of hydroxypropyl cellulose as precipitator inhibitor to the conventional SMEDDS led to the creation of supersaturable-SMEDDS [2]. Stability studies performed in simulated gastrointestinal fluids showed that S-SMEDDS maintained their physicochemical properties and hampered drug precipitation once in contact with intestinal basic pH. S-SMEDDS were not cytotoxic when in contact with Caco-2 cells and were able to open tight junctions, increasing epithelial permeability in a transient manner. Finally, *in vivo* pharmacokinetic highlighted that S-SMEDDS prolonged drug plasmatic circulation time compared to free drug and to conventional SMEDDS improving drug absorption.

Conclusion: Overall, by combining the attributes of SMEDDS together with the supersaturable characteristics, S-SMEDDS proved to be a successful strategy for the oral delivery of lipophilic drug molecules.

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Characterising cell internalization effects on the heat released by iron oxide nanoparticles

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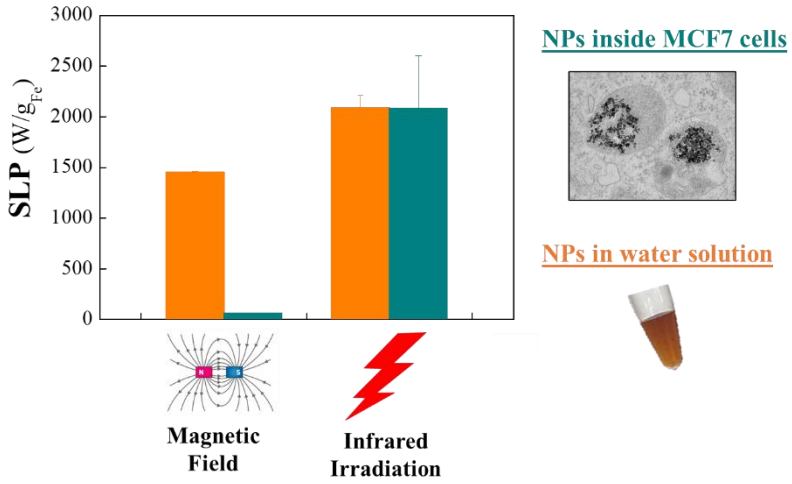
Background: Heating mediated by nanoparticles has become an efficient and minimally invasive therapy to treat solid tumors based on the local heat released by magnetic or plasmonic nanoparticles when subjected to alternating magnetic fields (Hac) and/or optical irradiation. Among those nanoparticles, iron oxide nanoparticles (IONPs) are promising nanomaterials employed in different therapeutic, diagnosis or imaging applications thanks to the precise control of synthesis, and high biocompatibility. Recent works show that IONP magnetic heat losses in biological environments are strongly reduced due to agglomeration and/or immobilization effects [1] [2]. However, the optical heating released by IONPs seems to be less influenced into biological matrices. The difficulties of IONPs to release a predictable or known magnetic heat dose into cells or tissues are being one of the main limitations for their clinical use. Then, it is mandatory to establish methodologies and determine the heat dose released by IONPs into biological matrices.

Experimental: In this work, we have compared the heating capacity of 20 nm high-quality iron oxide nanoparticles (IONPs) in water solution and inside live cells, through the determination of the temperature increments, the specific loss power (SLP) and the heat dose (μJ/cell). To do this, MCF7 cell line was incubated with 50 ug/mL of commercial IONPs (Magnetite PVP, Nanocomposix) for 3 hours. After 24 hours, cells were detached, and cell pellets were resuspended in DMEM media and subjected to H_{ac} (115 kHz, 28 kA/m) or near infrared radiation (NIR-0,8 W/cm^2, 808 nm). Iron content inside cell solutions was determined by ICP-OES and IONPs water solutions at the same iron

concentration were subjected to the same H_{ac} and NIR conditions. Moreover, equivalent protocol was performed with smaller IONPs (11 nm, FluidMag-CT, Chemicell). We have developed calorimetry and magnetometry methods to accurately determine SLP values under Hac or NIR.

Results: We observed outstanding optical SLP values (up to 2000 W/g_{Fe}) in high-quality magnetite-PVP IONPs under moderate irradiation conditions (808 nm and 0.3 W/cm^2). Furthermore, the intracellular and colloidal SLP values are similar, contrary to magnetic ones (see figure below), which shrink 100-folds. Nevertheless, 11 nm FluidMag-CT IONPs show much lower SLP values than the PVP IONPs. However, heat losses remain invariable inside live cells under H_{ac} or NIR. Photothermal heating of both FluidMag-CT and Magnetite-PVP IONPs have a cytotoxic effect on MCF7 cells, reducing their viability between 20-40%.

Conclusion: The invariability of optical losses released by IONPs represents a remarkable advantage for therapeutic applications in comparison to magnetic ones. Specially to quantify the intracellular optical heat losses, which can be by simply determined in IONPs dispersed in solutions under same experimental conditions (iron content and irradiation). Altogether, our results highlight the importance of proving the heating capacity of IONPs in biological environments in order to assess their efficacy as thermal agents for treating solid tumors. The quantification of intratumoral heat dose will precisely allow to correlate thermal effects to tumor response.



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Unravelling the chemotherapeutic and immunomodulatory effect of glioblastoma-targeted nanoparticles through a novel tumor niche-recapitulating 3D spheroid construct

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Background: Glioblastoma (GBM) is the most common and lethal type of primary brain tumor. The 5-year survival of GBM patients is still limited to a dismal 5%, highlighting the need to advance more effective GBM therapies. GBM tissue presents an abnormal expression of the L-type amino acid transporter 1 (LAT1), for which histidine (His) is an inexpensive and powerful targeting ligand [1]. Although His is expected to provide higher accumulation of drug nanoparticles (NPs) into GBM cells via LAT1 binding, consequently enhancing the anti-tumor response, it has been poorly explored in GBM-targeted therapies. Thus, this project proposes GBM-targeted, His-functionalized polymeric NPs loaded with docetaxel as a therapeutic with capacity to induce immunogenic cancer cell death [2]. On the other hand, a recent paradigm shift in the field of GBM immune environment revealed that the majority of tumor-associated macrophages in GBM are infiltrating bone marrow-derived

macrophages, and not microglia [3]. Therefore, the herein project also aims at providing a first-time developed donor-isolated macrophage/GBM crosstalk 3D spheroid construct to simultaneously study drug chemotherapeutic and immunomodulatory effects.

Experimental: Carbodiimide and carbamate hydrolysis chemical strategies were employed to synthesize a polymeric conjugate based on poly(lactic-co-glycolic) acid (PLGA) and His-functionalized polyethylene glycol (PEG), to serve as the NP core and shielding, respectively. The PLGA-PEG-His polymeric conjugate was characterized by various techniques such as NMR, optical contact angle measurements, FTIR and MALDI-TOF MS. The PLGA-PEG-His polymeric conjugate was further used to manufacture docetaxel-loaded NPs, through a previously established microfluidic technique of high reproducibility and easy scaling up [4]. Docetaxel-loaded PLGA-PEG-His NPs were fully characterized for physicochemical properties. Regarding the 3D spheroid construct, agarose micro-molds were used for high-throughput spheroid assembly. GBM cell binding of unloaded PLGA-PEG-His NPs was evaluated by flow cytometry in different cell lines (U251, U373, U87) to select the best cell model for the spheroid construct core. Human monocytes were isolated from healthy blood donor buffy coats provided by São João Hospital (Portugal). The 3D spheroid construct was optimized for the optimal total cell density (2500, 5000 and 1000 cells/spheroid) and tumor cell:monocyte percentage (50:50, 35:65, 20:80, 5:95), and visualized by microscopic techniques using H&E staining and immunohistochemistry. A preliminary assay was run to investigate the chemotherapeutic effect of docetaxel-loaded PLGA-PEG-His NPs compared to the free drug control.

Results: The chemical synthesis of the PLGA-PEG-His polymeric conjugate achieved 90% conjugation efficiency, as demonstrated by NMR; optical contact angle measurements indicated an intermediate PLGA-PEG/His hydrophilicity for the conjugate; FTIR confirmed an amide formation; MALDI-TOF MS revealed an unique ionization profile for the conjugate compared to the PLGA-PEG and His controls. Docetaxel-loaded PLGA-PEG-His NPs demonstrated scale-independent 250 nm size, 0.2 polydispersity index, 70% drug entrapment efficiency and a controlled drug release over 48 h. The GBM cell binding of unloaded PLGA-PEG-His NPs was 2.5-times higher than non-His-functionalized NPs in all tested cell lines. Regarding the 3D spheroid construct, U251 was selected as the tumor cell model, no H&E necrosis was observed in all tested total cell densities, and only the 50:50 and 35:65 tumor cell:monocyte percentage conditions assembled into a spheroid.

Immunohistochemistry revealed the spatial distribution of tumor-associated vimentin, extracellular matrix fibronectin and CD68 macrophage marker within the 3D spheroid construct (Fig. 1A). Docetaxel-loaded PLGA-PEG-His NPs drastically disturbed the morphology of the spheroid tumor core, suggesting a significantly higher level of cytotoxicity compared to the same dose of the free drug control (Fig. 1B).

Conclusion: This work has allowed the exploitation of His functionalization to synthesize cost-effective GBM-targeted NPs with capacity to undergo a significantly higher accumulation within tumor cells and disrupt the tumor core of a first-time proposed donor-isolated macrophage/GBM crosstalk 3D spheroid construct. Ongoing work is expected to open avenues regarding the immunogenic properties of the docetaxel-loaded PLGA-PEG-His by studying spheroid macrophage M1/M2 polarization.

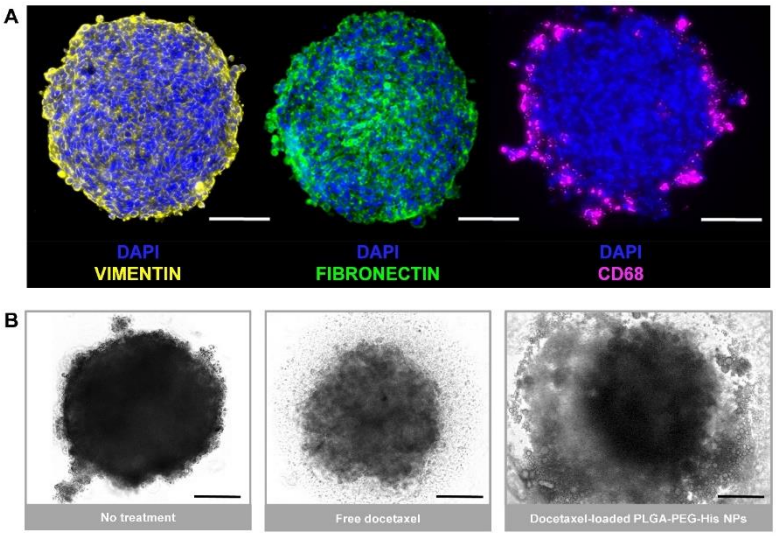

Figure 1 – (A) Spatial distribution of vimentin, fibronectin and CD68 within the 3D spheroid construct. (B) Impact of free docetaxel and docetaxel-loaded PLGA-PEG-His NP treatment on the morphology of the spheroid tumor core.

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Evaluation of nano-bio interactions of doxorubicin-coated gold nanoparticles

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Background:Nanomedicine is a rapidly evolvingfield that promises improved therapeutic efficacy and reduced toxicityof medicines. [1] Conjugation of a drug onto gold NPs (AuNPs)may alter the pharmacokinetics and pharmacodynamics and mediate its toxicity. [2] Doxorubicin (DOX), a cytotoxicdrug with a broad antitumor activity, was used as model compound to test the nano-drug delivery system. The aim of the study was to investigate and compare nano-bio interactions of newly synthesized AuNPs stabilized with biocompatible polyethylene glycol (PEG) and functionalized with DOX (DOXAuNPs), and AuNPs just stabilized with PEG (PEGAuNPs)

Experimental:Characterizationand stability assessmentof NPs included determination of size, size distribution, shape and zeta potential.Stability was evaluated in cell culture media(CCM) in the absenceand presenceofbovine serum albumin(BSA).Binding affinitiesand secondary changes in protein structureupon NPs and BSA interactionshave been investigated using fluorescencequenching and circular dichroism (CD) methods.The uptake of the AuNPswas studied using confocal microscopy.Acellular reactive oxygen species (ROS) production was studied by the DCFH-DA assay.

Results:Stability assessment showed increase in sizeand zeta potential forboth typesof NPs in CCMwhich indicated their destabilization. However, this was not observed in the presence of BSAdue to formation of protein corona. Binding affinities were almost similar between BSA and both typed of NPs. However, PEGAuNPs caused the higher conformational changes in BSAcompared to DOXAuNPs. The confocal microscopy images indicated stronger uptake ofDOX-AuNPs compared

to PEG-AuNPs.Finally, a dose-dependent ROS generation was determined for both NP types, with significantly larger effect shown in PEG-AuNPs.

Conclusion:The prepared DOX-AuNPs maintaintheir stability in biomimetic media by associating with BSA, while minimally affecting its structure. They are successfullytaken into cells, and show limited potential for oxidative stress.The results demonstrate good promise for their successful implementation as drug nanocarriers.

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FIRST COST ACTION CA17140 Conference, Riga,October15-17, 2019Technology). This research was funded by the Spanish Ministry of Science, Innovation and Universities (APCIN action in the framework of the European EuroNanoMedII Project “NanoPlasmiRNA”), Spanish MINECO project BIO2017-84246-C2-1-R, and Gobierno de Aragón (Diputación General de Aragón–Fondo Social Europeo and Predoctoral contract to R.R.J.).TOPIC OF THE PRESENTATION:νCurrent techniques and recent advances in physicochemical characterization of nanodrugsoQuality control of nanopharmaceuticals and nano-drug delivery systems throughout the productionoNano-bio interactions governing the efficacy of nanodrugsoTechniques and methods for evaluation of drug loading and release from nanoformulations

On the biological activity of luminescent gold(I) organometallic complexes: chemical modifications and some insights on how they enter the cells

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Background: Gold based compounds form a new family of cytotoxic agents of current, great interest as anticancer drug candidates. In particular, gold-phosphine compounds were investigated after the antiarthritic drug auranofin (thiolate–Au-PEt_3complex) was found to have biological activity against different cancer cells. A series of auranofin analogues containing thiolate ligands were prepared, as well as bis(phosphine)Au(I), phosphine-gold-halides, and phosphine-gold-alkynyl complexes.[1]

Experimental: Due to our expertise on gold(I) organometallic complexes and the study of their luminescent properties (which are also of great interest from a biological point of view[1,2]) we are focused on the synthesis and characterization of gold(I) organometallic complexes (mainly water soluble). We have designed different series of complexes with modifications on their chemical structure (chromophore, phosphine, alkyl length chain,…)[3] and hybrid systems containing nanoparticles in order to analyze how these changes can affect and improve their biological activity.

Results and Conclusion: The observed data show that both ligands coordinated to the metal atom have a direct influence on the biological activity. Additionally, the interaction of the systems with nanoparticles may improve in some cases these properties.

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CEA-targeted Nanoparticles as novel Chemotherapy for metastatic Colorectal Cancer treatment

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Background: Introduction: Colorectal cancer (CRC) is one of the deadliest cancers, mainly due to metastases appearing. The severe toxicity of 5-FU chemotherapeutic regimes renders it unsuccessful with limited bioavailability and low tumor-specific selectivity. In order to provide an effective, controlled and targeted therapy, nanomedicine constitute a promising alternative. CEA is an overexpressed molecule in CRC, constituting an interesting candidate to target CRC cells. This work intends to develop and characterize an innovative nanosystem with tropism to CRC cells expressing CEA, carrying the small drug, 5-FU.

Experimental: Poly (lactic-co-glycolic acid) (PLGA) and poly (ethylene glycol) (PEG) co-polymers were chemically conjugated with the anti-CEA ScFv antibody. Polymeric NPs were produced by double emulsion and loaded with 5-FU. Physical-chemical properties were assessed by DLS and LDA. Morphology, drug loading (DL) and conjugation efficiency (CE) were evaluated by TEM, NMR and HPLC, respectively. CEA expression in different CRC cell lines was evaluated by flow cytometry.

Results: The NMR spectrum of the polymer conjugated with anti-CEA scFV revealed characteristic peaks of groups from the PLGA as well as the presence of anti-CEA scFV peaks. Moreover, the peak of maleimide group in the polymer that was not submitted under the reaction was found. Moreover, 80% of CE was achieved. NPs with 165 nm were attained, with about PdI 0.2, confirming the monodisperse population and around 4% of DL. The surface charge was close to the neutrality (-2.8 ±0.2 mV) and the spherical shape was confirmed. Additionally, LS174T was found the cell line with more expression pf CEA and SW480 with no expression. Further, in vitro studies to assess binding efficiency and targeting ability of the NPs against CEA-expressing and non-expressing cells will be performed.

Conclusion: The conjugation of the PLGA-PEG-scFv polymer demonstrate the successful chemical conjugation. Moreover, through this work, we developed and characterized 5-FU-loaded NPs with satisfactory results in 5-FU encapsulation.

Ackonowledgments This work was supported by FCT - Fundação para a Ciência e a Tecnologia/ Ministério da Ciência, Tecnologia e Ensino Superior in the framework of the project "Institute for Research and Innovation in Health Sciences" (UID/BIM/04293/2019). This work is being performed within the scope of BiotechHealth PhD Program.

Diamond nanofilm normalizes proliferation and metabolism in liver cancer cells

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Background: Surgical resection of hepatocellular carcinoma is a frequent therapeutic procedure. However, a significant complication is a recurrence, which is associated with the degeneration of residual volume of the liver, as well as the presence of residual cells after resection. The aim of our research was to assess the possibility of using a biocompatible nanofilm, made of a colloid of diamond nanoparticles, to fill the side after tumour resection and optimize its contact with proliferating liver cells, minimizing their cancerous transformation. As a non-toxic, mechanically and chemically active material, diamond nanofilm (nfND) could be used to fill the cavity after liver tumour resection, creating an environment conducive to niche colonization and tissue regeneration.

Experimental: Experiments were performed using an in vitro method with HepG2 and C3A liver cancer cells and HS-5 non-cancer cells. An aqueous colloid of diamond nanoparticles, which covered the cell culture plate, was used to create the nanofilm. The roughness of the resulting nanofilm was

assessed using atomic force microscopy. Metabolic mitochondrial activity and cell proliferation were measured using the XTT and BrdU assays. Cell morphology and a scratch test were used to evaluate the invasiveness of cells on the nanofilm. Flow cytometry was used to determine the number of cells in particular phases of the cell cycle. Analysis of changes in protein expression in HepG2 and C3A cells due to the application of nfND was performed using mass spectrometry.

Results: The nfND, dedicated to covering the tumour resection cavity, created a surface with increased roughness and exposed oxygen groups compared with a standard plate. All cell lines were prone to settling on the nanofilm, and the HepG2 and C3A cancer cells formed more relaxed cell clusters indicative of individual migration. The surface compatibility was dependent on the cell type and decreased in the order C3A >HepG2 >HS-5. Although invasion was reduced in two cancer lines, the nanofilm had the greatest effect on the C3A line, reducing proliferation and increasing the G2/M cell population. Proteomic analysis showed statistically significant changes in the expression of 189 HepG2 cell proteins and 172 C3A cell proteins after culturing with nfND. Among the proteins with altered expression, membrane (HepG2) and nuclear (C3A) proteins dominated. In addition, cancer cells demonstrated upregulation of many key proteins in the respiratory chain, including ATP synthase.

Conclusion: In vitro studies demonstrated the antiproliferative properties of a nanofilm made of diamond nanoparticle colloid against C3A liver cancer cells. At the same time, the need to personalize potential therapy was indicated due to the differential protein synthetic responses in C3A vs HepG2 cells. We documented that nfND is the source of signals capable of normalizing the expression of many intracellular proteins involved in the transformation to non-cancerous cells.

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Graphene oxide as a biocompatible surface coating – activity comparison of bare flakes and modified with silver nanoparticles

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Background: Graphene oxide (GO) is an oxidized form of graphene, the thinnest material in the world. Oxidation of graphene leads to significant increase of hydrophilicity and decrease of the toxicity at the same time. GO can be easily modified with bioactive agents, drugs or nanoparticles, allowing for an equal and slow release of a drug or ions from the attached nanoparticles, such silver nanoparticles (AgNP). It makes GO a very interesting candidate for medical applications, such as innovative antibacterial coatings or dressings.

Experimental: We performed *in vitro* experiments on two cell lines representing cells in lung and skin (A549, HFFF-2, respectively), comparing the cytotoxicity of GO, AgNP and a composite prepared from GO and AgNP. We compared the cytotoxic activity of the nanomaterials in different concentrations (ranging from 1 to 25 ppm for GO and 1 to 5 ppm for AgNPs) by a classic approach introducing the nanomaterials to the culture medium, as well as by coating the cell culture pre-treated surfaces.

Results: Lung cell line showed higher sensitivity to the nanomaterials than skin cells. For all tested nanomaterials we observed the decrease in mitochondrial activity in both cell lines, demonstrated by MTT test, however neutral red uptake and LDH release tests revealed a decrease of viability only in A549 cell line, while BrdU proliferation assay showed a decrease only for the GO-AgNPs composite. Introducing the nanomaterials into the culture media decreased the number of cells, however cells showed an excellent growth on surface coated with GO and GO-Ag composite. Moreover, prolonged incubation for 7 days revealed that skin cells had high affinity to GO and growth preferably on GO.

Conclusion: GO in low concentration is a biocompatible, suitable candidate for innovative biomaterials, improving the distribution of the AgNPs or other agents, thus prolongating their activity and prevent the accumulation of the active agent in one site.

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In vitro exploration of the synergistic effect of alternating magnetic field mediated thermo-chemotherapy with doxorubicin loaded dual ph- and thermo-responsive magnetic nanocomposite carriers

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Background: Nanoparticle induced hyperthermia has been considered as a promising approach for cancer treatment for decades. The local heating ability and drug delivery potential highlight a diversified possibility in clinical application, therefore a variety of nanoparticles that has been developed accordingly. However, currently, only a few of them have been translated into clinical stage indicating the 'nanoparticle medically underserved' situation, which encourages their comprehensive biomedical exploration.

Experimental: This study presents a thorough biological evaluation of previous well-developed dual pH- and thermo- responsive magnetic doxorubicin-nanocarrier (MNC-DOX) in multiple cancer cell lines. The cytoxicity of the nanocomposites has been determined by the MTT assay on primary cell lines. The histology and fluorescence microscopy imaging revealed the efficiency of various cellular uptake of nanocarriers in different cell lines.

Results: The IC_{50} of MNC-DOX is significantly higher than free DOX without alternative magnetic field (AMF), which implied the potential to lower the systemic cytotoxicity in clinical research. The concurrent thermo-chemotherapy generated by this platform has been successfully achieved under AMF. Promising effective synergistic results have been demonstrated through in vitro study in multi-model cancer cell lines via both trypan blue exclusion and bioluminescence imaging methods. Furthermore, the two most used magnetic hyperthermia modality, namely intracellular and extracellular treatments have been compared on the same nanocarriers in all 3 cell lines, which showed treatment after internalization is not required but preferable.

Conclusion: These results lead to the conclusion that this dual responsive nanocarrier has extraordinary potential to serve as a novel broad-spectrum anticancer drug and worth to be pursued for potential clinical applications.

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POSTER PRESENTATIONS

pH-sensitive nanoparticles of amphiphilic triazine-carbosilane dendrons for drug delivery

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Background: the use of nanoparticles as a platform for drug delivery has received a lot of attention to their ability to improve the therapeutic properties of cargo compounds. The drugs incapsulated in nanoparticles are protected from the components of the biological environment, exhibit greater selectivity and efficiency of penetration into target tissues, while the toxic effect is reduced. Moreover, of particular interest are stimulus-sensitive nanoparticles, which change their structure with changes in pH or temperature, fermentation medium, etc.

Experimental: new amphiphilic molecules — triazine-carbosilane dendrons have been synthesized. Organic synthesis of molecules was carried out in several stages. The critical concentration of micelle formation of nanoparticles was calculated by detecting the fluorescence of the encapsulated pyrene. Method of dynamic light scattering was used to determine the size of nanoparticles. The encapsulation efficiency was calculated by comparing the optical absorption spectra. Dialysis was performed against water on semipermeable membranes MWCO 3500 to remove excess therapeutic molecules.

Results: the critical micelle concentration was found, at which the synthesized molecules self-assemble into nanoparticles ~100 nm in diameter. Doxorubicin, methotrexate, 5-fluorouracil can be placed inside these nanoparticles by a simple procedure. With a decrease in pH to 6, the nanoparticles are reorganized and the encapsulated drug is released. Also, these dendrons form a complex with therapeutic microRNAs and siRNAs. **Conclusion**: we proposed a simple method for obtaining nanoparticles based on carbosilane amphiphilic dendrons, self-organizing in solution. Experiments have proven the potential of the nanoparticles as a platform for the delivery of therapeutic molecules.

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Effective intracellular delivery of bevacizumab via PEGylated polymeric nanoparticles targeting the CD44v6 receptor in colon cancer cells

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Background: Colorectal cancer (CRC) is the third most common cancer and the second most deadly cancer worldwide making the research for new therapies essential and urgent. The first-line treatment for metastatic CRC (mCRC) consists of chemotherapy. including fluoropyrimidine (intravenous 5-fluorouracil (5-FU) or the oral capecitabine) (1). However, this therapy is often associated with severe side effects since it does not target a molecule specifically, acting also against the healthy tissues and cells. Bevacizumab (Avastin®) is a monoclonal antibody (mAb) that interacts directly with extracellular vascular endothelial growth factor (VEGF) and was the first angiogenesis inhibitor to be approved by the Food and Drug Administration (FDA) in 2004 for the treatment of advanced colon cancer (2). However, an antibody-based therapy has several challenges, including tumor tissue penetration and the mode of action of the drug. The major limitation is attributed to drug distribution since only a low percentage of the administered dose of mAbs with large size can reach the tumor due to the "binding site barrier effect". This type of treatment also requires multiple administrations, resulting in high doses and an expensive therapy. The use of nanoparticles (NPs) for bevacizumab delivery overcomes some limitations of antibody-based delivery, allowing the modulation of the

release profile of bevacizumab and potentiates its intracellular delivery to cells (3). The cluster of differentiation 44 containing exon 6 (CD44v6) overexpression has been described to play a major role in CRC metastatic behavior, being an independent factor that inversely affects the survival of CRC patients. CD44v6 is a membrane adhesion molecule, associated with the activation of different signaling pathways involved in cancer progression, representing a diagnosis and therapeutic target for CRC (4). The aim of this study was the development of a nanomedicine to target specifically CD44v6-overexpressing cells to improve CRC treatment (5). It involves the production of functionalized NPs with a ligand specific for CD44v6 and loaded with anti-VEGF mAb bevacizumab. To achieve an active targeting to CRC cells, NPs were then functionalized with a well-characterized human antibody fragment (Fab) specific to human CD44v6, AbD15179. The system aimed the intracellular delivering of bevacizumab through the interactions of NPs with the CD44v6 cell-surface receptor overexpressed in CRC cells.

Experimental: Aiming at a targeted therapy to colorectal cancer cells, the anti-VEGF mAb bevacizumab was loaded into poly(lactic-co-glycolic acid)-polyethylene glycol (PLGA-PEG) NPs functionalized with a human antibody fragment (Fab) specific for CD44v6-expressing human cancer cells. The NPs were characterized physically and technologically, and their cytotoxicity, binding specificity and affinity to CD44v6 were studied in cancer cells. To understand the biological effect of NP targeting, the intracellular levels of bevacizumab and VEGF were evaluated after the incubation of targeted and untargeted NPs.

Results: The sizes of NPs were in the range of 150–250 nm, a PdI between 0.1 and 0.25, and a negative charge between −5 and −10 mV, with an association efficiency and drug loading of bevacizumab of 86.5 ± 1.8% and 7.9 ± 0.2%, respectively. Cell toxicity studies showed absence of cytotoxicity for all PLGA-PEG NPs in both types of CRC cells. v6 Fab-PLGA-PEG NPs containing bevacizumab specifically bonded to the CD44v6 cell surface receptor and exhibited higher internalization into CD44v6+ epithelial cells than bare and (−) Fab-PLGA-PEG NPs. The intracellular levels of bevacizumab were significantly higher in cells incubated with v6 Fab-PLGA-PEG NPs and these NPs resulted in a significant decrease in the intracellular VEGF compared to untargeted NPs and free bevacizumab.

Conclusion: Overall, NPs demonstrated adequate physical and technological characteristics, did not show cytotoxicity at the concentrations tested and NPs decorated with v6 Fab seemed to bind specifically to CD44v6 on the surface of cells, with a lower binding to cells that do not express CD44v6. PLGA-PEG NPs, surface-functionalized with a v6-specific Fab, have the potential to intracellularly deliver bevacizumab into CD44v6 expressing cancer cells.

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Physicochemical Characterization of Oligonucleotide Conjugated Silica Nanoparticles for Drug Delivery Systems

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Background: During recent years, nano-drug delivery systems are highly preferred due to the controllable and targeting properties [1]. Carrier components are vital for drug delivery systems. Mobil Composite Material Number-41 (MCM-41) types of silica nanoparticles are suitable drug carriers due to their gold like properties such as high surface area, high thermal stability, high hydrophobicity, and functional surface [2]. Mesoporous structure of MCM-41 has ability to enclose drug molecules then release it in response to stimulation [3]. Synthesizing a nano-drug, especially characterization step is a complex process. On the other hand, measuring the physicochemical properties of a material is crucial to conduct study in a better controlled condition. In this context, multi-method approaches have benefits to characterize the nanoparticles, such as measuring size and zeta potential simultaneously. Therefore, aim of the present study is to characterize MCM-41 nanoparticles for the first step of a single strand oligonucleotide (aptamer) conjugated drug releasing system with a multi-method approach.

Experimental: After synthesis of MCM-41, the size and charge of the nanoparticles were measured using Dynamic Light Scattering (DLS) and Electrophoretic Light Scattering (ELS) in acidic pH. The area of MCM-41 pores was analyzed by Brunauer–Emmett–Teller (BET). Atomic Force Microscopy (AFM) and Transmission Electron Microscopy (TEM) were used to analyze the shape and surface topography of the nanoparticles. Lastly, amine functionalization for aptamer bindings on the nanoparticles was measured using Fourier-transform infrared spectroscopy (FTIR).

Results: TEM and AFM images exhibited the surface topography and morphology of the nanoparticles. The size of nanoparticles was 287 nm, which was in the nano-range, and BET analysis showed that the average pore size was 2.83 nm. The surface charge of the particles has been determined by zeta potential, which was -23.4 mV in acidic pH. Utilizing this surface charge, positively charged (3-Aminopropyl)-triethoxysilane molecules were added to the negatively charged MCM-41 nanoparticles as a linker for aptamer. The NH2 bonds in the nanoparticle structure was confirmed with FTIR analysis.

Conclusion: Multi-characterization methods may help to comprehend nanostructure-based systems and synthesis in a more controlled manner in order to perform better drug-release studies. The present study may pave the way for this purpose.

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Polymer Drug Conjugates for Pancreas Adenocarcinoma

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Background: Cancer is one of the most dangerous diseases defined as out of control cell growth. According to World Health Organization, 19.3 million cancer cases were diagnosed in 2020 and cancer led to 9.96 million deaths in 2020.[1] Pancreatic ductal adenocarcinoma (PDAC) among cancer types is the fourth leading cause of cancer deaths in spite of a low incidence. The five-year survival rate of PDAC, which is among the lowest of all cancer types, is under 5%.[2] This dramatic result is mainly caused by the lack of distinctive symptoms and credible biomarkers, which prevent early diagnosis.[3] Surgery, radiation therapy and chemotherapy are treatment options for pancreatic cancer. Unfortunately, less than 20 % of diagnosed patients may be cured via surgery because the cancer has spread into other parts of the body until it is diagnosed.[4] Furthermore, high local recurrence and chemoresistance caused by cancer stem cells (CSCs) are other major challenges for the treatment.[5] Although chemotherapy is successful to some extent, many disadvantages of chemotherapy drugs limit their bioavailability and applicability. Low water solubility, rapid clearance, short circulation time in blood stream, and nonspecific biodistribution are some disadvantages of anticancer drug. To overcome these limitations, polymeric drug carriers have gained an importance in the recent years.[6] By means of drug carrier, drug delivery profile can be adjusted to create desired therapeutic effect by avoiding from side effects and toxicity.

In this study, hydroxychloroquine (HCQ) and gemcitabine (GEM) were used as drug. GEM is an anticancer drug which interferes with DNA production. HCQ is commonly known as an anti-malaria drug but recently it has been shown that chloroquine (CQ) is an effective adjuvant therapy to chemotherapy because it offers more tumor elimination when it is used with GEM.[7] We synthesized hydroxychloroquine and gemcitabine containing polymer-drug conjugates (PDC) for pancreas

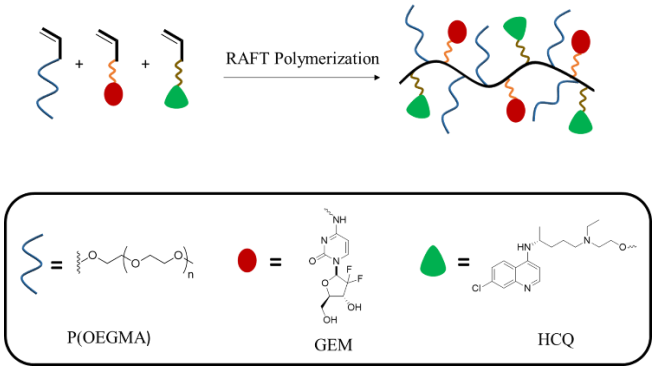
adenocarcinoma. The linker attaching the drug molecule to the backbone of the polymer has extensive effect on the release profile of the drug. The linkers for each drug molecule has been optimized for an effective release within the tumor and minimal release while circulating in plasma. Cytotoxicity experiments against BxPC-3, Capan-1, Panc-1 and MiaPaca-2 cells were performed to determine the differences between alternative nanomedicines carrying HCQ and GEM.

Experimental: Drug release profiles from copolymer were studied in pH 5.4, pH 7.4, plasma and enzyme and released drug amount was determined by LCMS analysis. Cytotoxicities of free drugs and polymer drug conjugate was investigated against pancreatic cancer cell lines (BxPC-3, Capan-1, Panc-1 and MiaPaca-2. Cell viabilities were determined via Cell Counting Kit-8 (CCK-8) assay.

Results:

Synthesis of Drug Conjugated Polymer-Drug Conjugate (PDC):

Drug conjugated polymer was synthesized by attachment of HCQ and GEM to polymer backbone. As a polymerization technique, reversible addition-fragmentation chain transfer (RAFT) polymerization was used to prepare HCQ-GEM conjugated polymer (P-HCQ/GEM) (Scheme 1). To provide sustained drug release, drugs were attached to polymer via pH sensitive linker. To take advantage of EPR effect, polymer with high molecular weight was synthesized.



Scheme 1. Fabrication of polymer-drug conjugate (PDC).

pH dependent release from drug attached polymer conjugate

GEM and HCQ release from PEGMA-HCQ/GEM copolymer was evaluated in pH:5.4, pH:7.4, rat plasma and enzyme. Copolymer solution was incubated in different pH media, rat plasma and enzyme. Drug release ratios were determined using LCMS. In the end of 144h, while GEM release reached into 100 % in enzyme 1-2 mixture, HCQ release reached into 98% in pH 5.4 (Figure 1).

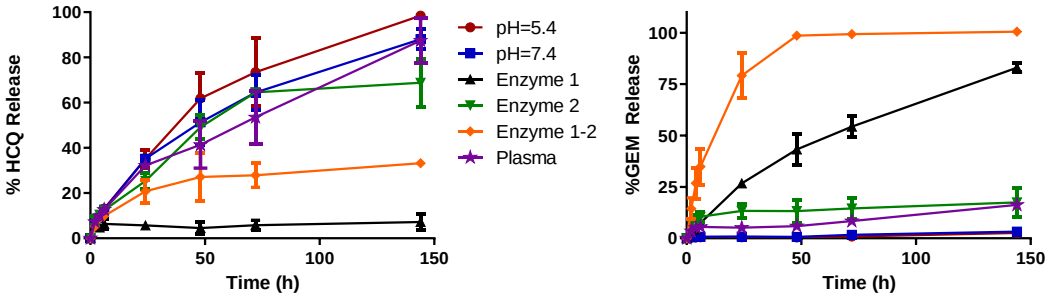


Figure 1. HCQ-GEM release profile from copolymer.

Effect of cytotoxic HCQ and GEM containing polymer conjugate was investigated on BxPC3, Capan-1, Panc-1 and MiaPaca-2 using CCK-8 assay. EC50 results were given in Table 1.

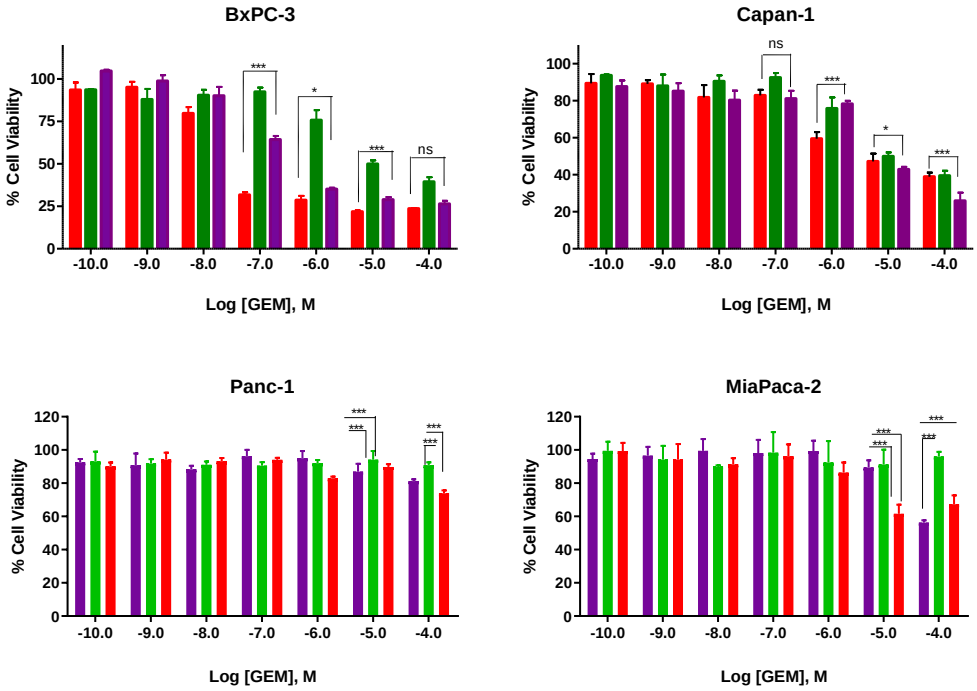


Figure 2. Cytotoxicity graphs of free drugs and polymer drug conjugate.

Table 1. EC50 results of free drugs and polymer drug conjugates on pancreas cancer cell lines.

EC50(μM)				
Compounds	BxPC-3	Capan-1	Panc-1	MiaPaCa-2
GEM	0.025	0.73	2.62	1.68
HCQ	2.47	2.58	180500	0.23
PGEM/HCQ	0.097	5.73	18.4	88.5

Conclusion: In this study, drug conjugated copolymer was synthesized via RAFT polymerization. Drug release profile from copolymer was demonstrated under different conditions. Cytotoxicity of copolymer was investigated on pancreatic cancer cell lines.

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An integrated approach for standardized guidelines and validation of physicochemical characterization of nanopharmaceuticals

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The field of nanomedicine utilizes nanomaterials to improve diagnosis, prevention and treatment of many diseases and cancer [1]. Physicochemical characterization techniques for nanocarriers play a key role in the assessment of nanopharmaceuticals' application for diagnostics and targeted drug delivery of anti-cancers to neoplastic cells/tissues. If diagnostic tools and therapeutic approaches are combined in one single nanocarrier, a new platform called nanobiotheranostic is created. Several analytical technologies are used to characterize nanopharmaceuticals and nanoparticles and their properties so that they can be properly used in cancer therapy. Currently there are no technical specific guidelines for physicochemical characterization of nanomaterials based on formulations for diagnostic or therapeutic use [2]. There is an urgent need for standardized protocols and procedures for the characterization of nanoparticles, especially those that are intended for use as cancer theranostics. Nanomaterials, including those with potential for clinical and biomedical applications, possess novel and emerging physicochemical properties that have an impact on their physiological interactions and body biodistribution, from the molecular level to the systemic level. There is a lack of standardized methodologies or regulatory protocols for detection or characterization of nanomaterials. Many methods have been used for evaluating manufactured nanomaterials, including techniques in optical spectroscopy, electron microscopy, surface scanning, dynamic light scattering, circular dichroism, magnetic resonance, mass spectrometry, X-ray scattering and spectroscopy, and zeta-potential measurements, as well as methods in the categories of thermal techniques, centrifugation, chromatography, and electrophoresis [3]. This work wants to provide an overview of the current state of the art and suggest potential combinations of physicochemical techniques that can provide advancements in the validation of physicochemical characterization of nanopharmaceuticals.

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Molecular imaging platforms based on magnetic iron oxide nanoparticles derivatives

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Background: Molecular imaging has witnessed over the last century, a revolution in terms of contrast agents development [1]. Magnetic iron oxide nanoparticles serve as a promising new platform for molecular imaging and attracted growing relevance due to their biocompatibility, stability, specificity, and potential applications in biomedicine, especially as magnetic resonance imaging (MRI) diagnostic vehicles [2]. The concept of functionalization proved to be an effective strategy for the stabilization of the nanoparticles in biological media, in order to avoid pre-targeting degradation and to enhance the biocompatibility of the nanoparticles [3]. Taken together, functionalized magnetic nanoparticles has the adequate features to provide insights into non-invasive diagnosis and disease progression, prognosis and response to therapy.

Experimental: In this paper, we report a strategy divided in several steps, for the development of potential MRI contrast agent candidates:

- first, magnetic nanoparticles were synthesized by co-precipitation method in the presence of a non-ionic surfactant, Pluronic F-127 using three different types of stirring (mechanical stirring, high-pressure homogenization (HPH) and ultrasonication (US));
- subsequently, the magnetic materials obtained using mechanical stirring were functionalized with silica and glucose chains;
- then, the structure and physicochemical properties of the functionalized magnetic iron oxide nanoparticles were studied by multiple methods;

- cell viability assays using MTT tests were performed on normal V79 cell line.

Results: The magnetic iron oxide nanoparticles derivatives contain magnetic iron oxide core, silica and glucose shell as confirmed by FT-IR spectroscopy, and have magnetic properties as showed by vibrating-sample magnetometer analysis. The functionalized magnetic materials were also characterized regarding their size distribution using dynamic light scattering, their morphology by transmission electron microscopy and their *in vitro* behaviour.

Conclusion: In summary, a simple and optimized method for preparing magnetic iron oxide nanoparticles conjugated with silica and glucose chains was developed. The obtained results for the optimum batch, confirm that these new designed molecular imaging probes can be used *in vivo* as MRI contrast agents.

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Genotoxicity assessment of TiO_2 nanoparticles in SH-SY5Y cells: suitability of the cytokinesis-block micronucleus test

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Background: Standard toxicity tests might not be fully adequate for evaluating nanomaterials since their unique features are also responsible for unexpected interactions. The *in vitro* cytokinesis-block micronucleus (CBMN) test is recommended for genotoxicity testing of pharmaceuticals intended for human use, but cytochalasin-B (Cyt-B) may interfere with nanoparticles (NP), leading to inaccurate results. Our objective was to determine whether Cyt-B could interfere with micronuclei (MN) induction by TiO_2 NP in human SH-SY5Y cells, as assessed by CBMN test.

Experimental: Cells were treated for 6 or 24 h, according to three treatment options: co-treatment with Cyt-B, post-treatment, and delayed co-treatment. Influence of Cyt-B on TiO_2 NP cellular uptake and MN induction as evaluated by flow cytometry (FCMN) was also assessed.

Results: TiO_2 NP were significantly internalized by cells, both in the absence and presence of Cyt-B, indicating that this chemical does not interfere with NP uptake. Dose-dependent increases in MN rates

were observed in CBMN test after co-treatment. However, FCMN assay only showed a positive response when Cyt-B was added simultaneously with TiO_2 NP, suggesting that Cyt-B might alter CBMN assay results. Still, no differences were observed in the comparisons between the three treatment options assessed.

Conclusion: Post-treatment and delayed co-treatment of Cyt-B, proposed by OECD (2014) for CBMN test when applied to nanomaterials, seem not to be adequate alternatives to avoid Cyt-B interference under the specific conditions employed in this study. Consequently, further investigations are necessary to define additional protocol alternatives of CBMN assay for accurately assessing genotoxicity of nanomaterials.

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Synthesis and development of phenanthroline-based derivatives to interact with the G-quadruplex motif present in human pre-MIR150

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Background: The human *MIR150* are significantly upregulated in Non-Small Cell Lung Cancer (NSCLC) and have been reported to have an important role in NSCLC development [1–2]. Based on that, the control of mature miR-150 production can provide a strategy to fight NSCLC development. The presence of alternative secondary structures in pre-miRNAs can affect their recognition and consequent processing by Dicer [3]. Recently, it has been reported that *pre-MIR150* folds into a G-quadruplex (G4) structure [4], which could regulate their levels, thus unveiling a new potential therapeutic strategy. G4 are non-canonical four-stranded secondary structures formed by G quartets arranged in a planar-square manner and connected by Hoogsteen bonds [5]. The formation of these G4 structures in the stem-loop region of pre-miRNAs interferes with Dicer activity and decreases mature miRNA production inside the cell [6].

Experimental: We have synthetized phenanthroline-based ligands with the aim of binding and stabilizing the G4 motif found in the region of *pre-MIR150*. The interaction of these ligands with the G4 motif has been evaluated using a combination of biophysical methods.

Results: We have synthetized and characterized twelve phenanthroline-based ligands. These ligands showed moderate activity in terms of thermal stabilization of the G4 motif present in *pre-MIR150*.

Conclusion: This study has explored the suitability of the synthesized molecules to interact with the G4 motif and provides invaluable information about the structural modifications that should be carried out in order to maximize their activity.

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The unpredictable protein corona interaction with Multiwall Carbon Nanotubes and a versatile functionalization technique

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Background: The intrinsic physicochemical properties of multiwall carbon nanotubes (MWCNTs) such as elemental composition, resilience, thermal properties, surface reactivity, and in particular the ability to capture biomolecules on their surface make them the undisputed interest in biotechnology[1,2]. The protein's interaction with MWCNTs creates a biological coating that endows them the ability to interact with some cell receptors, penetrate membranes or interfere with cell biomechanics, so controlling the biocorona is pivotal in MWCNTs nanobiotechnology.

Experimental: MWCNTs functionalization was carried out with different human serum under several conditions. It was evaluated by TEM, AFM and TGA techniques and also examined by SDS–PAGE protein analysis. Finally, for a versatile functionalization technique, a recombinant gene was synthesized and the protein was expressed and purified. Then MWCNTs were functionalized with this protein.

Results: We demonstrate a significant increase in CNTs diameter after protein functionalization and also that between 20 and 60% of the mass of functionalized nanotubes corresponds to protein, with single-walled CNTs capturing the highest amounts.

We also analyze the biochemical "landscape" of the proteins captured by the different nanotubes after functionalization under various conditions. This study revealed a significant variability of the proteins in the corona as a function of the type of nanotube, the functionalization temperature, or the time after exposure to serum. Due to the unpredictable assortment of proteins captured by the corona and the biological implications of this biocoating, we finally designed a method to genetically engineer and produce proteins to functionalize nanotubes in a controlled and customizable way.

Conclusion: We demonstrate the high unpredictability of the spontaneous protein corona on MWCNTs and propose a versatile functionalization technique that prevents the binding of nonspecific proteins to the nanotube to improve the use of MWCNTs in biomedical applications, for example as drug nanocarrier.

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Long-term biodistribution of gold nanospheres *in vivo* in mouse

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Metal-based nanoparticles are a promising tool in biomedical applications. Gold nanoparticles (AuNPs), as wide studied nanomaterials, are suitable candidates for e.g. cancer treatment by drug delivery, imaging, photothermal therapy or gene therapy [1,2]. But for each new treatment agent it is important to monitor its fate in the organism. Importantly, the drug should be safely eliminated from the body. On the other hand, specific accumulation of nanoparticles in organs could be utilized by targeted therapy.

In our study, sphere shaped AuNPs with 10 nm in diameter, coated with bovine serum albumin (BSA) were applied to C57BL/6 by systemic administration. Mice were regularly weighted and after 120 days liver and spleen were extracted and analyzed by atomic absorption spectrometry.

Our results show, that application of nanoparticles did not affect health status of the mice for the duration of the experiment, despite AuNPs were still detectable in liver and spleen 120 days after nanoparticles administration. Both organs in the treated group were slightly heavier than in control group. Whether this could be a direct effect of accumulated AuNPs is the object of further investigation. As AuNPs can be potentially cleared by the liver as the main excretory organ for particles above the renal filtration limit and by splenic clearance, the results indicate, that AuNPs accumulate predominantly in sites of excretion and persist there for at least 4 months.

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Cell-based toxicity evaluation of biocompatible multifunctional nanodevices for cancer nanomedicine

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Background: Polymeric nanoparticles offer a great flexibility adapting its chemistry composition, size, stability, morphology and surface functionality. As a result, they are used in Biomedicine as drug delivery systems and diagnostic agents for a wide range of applications in diagnosis, therapy and theranostics [1,2]. Recently, we have designed several polymeric nanoparticles for selective drug delivery, theranostic and sensing [3-5]. Herein we reported the results of the preclinical evaluation of these multifunctional nanodrugs, focusing on running toxicity assays and cellular uptake mechanisms protocols.

Experimental: Two different cancer cell lines (breast cancer MDA MB 231, and lung cancer A549) have been interrogated.

Toxicity assay protocols: 1) [^{3}H]-thymidine incorporation. Direct measures of proliferation are achieved using the thymidine incorporation assay. This strategy is based on a labelled nucleoside, 3H-thymidine, that is incorporated into new strands of chromosomal DNA during mitotic cell division then the extent of cell division that has occurred can be determined. 2) Protein synthesis assay. Direct measures of cell viability are achieved using the leucine incorporation assay. This strategy is based on a labelled amino acid, [^{3}H]-leucine, that is incorporated into new proteins during cell activity. If cells are suffering cytotoxic effects or stress responses, the synthesis of new proteins could be affected.

Cellular uptake mechanism assays protocols: In order to find out the mechanism of internalization of the nanodevices, immunostaining was done with the same two cell lines, after 24 hours of incubation with nanoparticles. 1) Lysosomes staining. Lysosomes were labelled with LysoTracker for tracking them after nanodevices internalization. 2) Late endosomes staining. This type of endosomes, that mediate endocytosis, were labelled with anti-CD63 antibody and a fluorescent-labelled secondary antibody for tracking them after nanodevices internalization.

Results: Three nanodevices were studied: (i) a non-engineered nanoparticle which is amino-functionalised (NK-NP), (ii) a fluorescent-labelled nanoparticle (using a far red fluorophore, Cy5) (Cy5-NP), and (iii) a nanoparticle loaded with a standard antitumoral drug (doxorubicin-DOX) (DOX-NP). Nanoparticles without functionalization (NK-NPs) and fluorescently labelled nanoparticles (Cy5-NPs) did not induce any cytotoxic effects in both studied cell lines. After 24 hours of incubation, cells did not alter their DNA and protein synthesis, as they have the same activity level as untreated cells.

However, DOX-NPs induced cytotoxic effects on cells. After 24 hours of incubation, both cell lines showed a decrease in DNA synthesis activity due to DOX genotoxic effect. However, protein synthesis activity did not seem to be affected by DOX-NPs internalization.

About the internalization assays: by confocal microscopy analysis, no colocalisation of these large nanoparticles with the stained lysosomes and late-endosomes were observed. This suggests that the NPs internalized by endocytosis accumulated in early endosomes or macropinosomes and did not further mature or fuse into late endosomes and lysosomes. Alternatively, these NPs were not internalized by endocytic pathways.

Conclusion: Cytotoxic evaluation has confirmed the innocuousness of these polymeric nanodevices. Remarkably, only nanoparticles loaded with an antitumoral drug showed cytotoxic effect. After 24 hours of incubation, DOX-NPs decreased DNA synthesis activity levels in cells, demonstrating the genotoxic effect of doxorubicin and the efficient drug release of the nanodevice. This *in vitro* validation was successfully achieved in two different cancer cell lines.

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ICG-tagged aptamer as drug delivery system for malignant melanoma

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Background: Malignant melanoma accounts for about 1% of all skin malignant tumors and represents the most aggressive and lethal form of skin cancer [1]. Clinically, there are different therapeutic options for melanoma treatment, such as surgery, chemotherapy, radiotherapy, photodynamic therapy and immunotherapy [2]. However, serious adverse effects usually arise, and survival rates are still low because a high number of patients present relapses within 6–9 months after therapy. AS1411 is a G-quadruplex (G4) aptamer capable of tumor-specific recognition, since it binds to nucleolin, a multi-functional protein expressed in many different types of cancer cells [3].

Experimental: We present a novel drug delivery system composed of AS1411 and indocyanine green (ICG) to track its accumulation in a mouse model of melanoma. Using a simple supramolecular strategy, we conjugated the complex AS1411-ICG with C_8 ligand, an acridine orange derivative with potential anticancer ligand. Then, we performed *in vitro* cytotoxicity experiments using the B16 mouse melanoma cell line, and *in vivo* experiments using a B16 mouse melanoma model to study biodistribution and histological changes.

Results: The circular dichroism data suggest that C_8 does not affect the parallel G4 topology of AS1411-ICG, whereas it increases its thermal stability. Incubation of B16 melanoma cells with the AS1411-ICG complex associated with C_8 increases the cytotoxicity compared with AS1411-ICG alone. From the *in vivo* studies, we conclude that both AS1411-ICG and AS1411-ICG-C_8 presented the potential to accumulate preferentially in tumor tissues. Moreover, C_8 seems to be efficiently removed from the mice's bodies through kidney clearance.

Conclusion: These results suggest that these complexes derived from AS1411 aptamer could act as a delivery system of ligands with antitumoral activity for melanoma therapy.

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Synthesis of a Dual Prodrug for Pancreatic Ductal Adenocarcinoma

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Background: Although pancreatic cancer accounts for approximately 5% of all cancers, it remains one of the most dangerous. Around 54% of cases are diagnosized in the fourth stage and the five years survival rate for pancreatic cancer didn't reach 10% till 2017.[1] Pancreatic ductal adenocarcinoma (PDAC) is the most common malignancy of the pancreas. The most effective way of treatment is the chemotherapy despite all its disadvantages. In the context of preparation of chemotherapy agents against the PDAC, our work consists on the synthesis of PEGylated prodrugs based on the combination of hydroxychloroquine (HCQ) and gemcitabine (GEM). GEM monotherapy is the standard front-line therapy for patients with PDAC and HCQ, an antimalarial drug, recently proved its efficiency to kill stem cells in PDAC.[2] Although GEM has the potential to be used as a single agent in many of the carcinomas, the most common regimen in PDAC is called FOLFIRINOX: a combination therapy, consisting of 5-fluorouracil (5-FU), irinotecan, oxaliplatin along with GEM.[3] Just like many other chemotherapy agents GEM is also subjected to resistance by PDAC cells, a serious problem that needs to be tackled.[4] Combination of two drugs in a nanomedicine format has the potential to address both the issue of combination therapy and chemoresistance. The cytotoxicity of the synthesized prodrugs was examined for BxPC-3, Capan-1, Panc-1 and PSC stellate cell lines and the prodrug decomposition studies to yield the original drugs were performed.

Experimental: Drug release from PEGylated drug conjugates were determined via LC-MS. The drug containing PEG solutions were prepared with total concentration of 1 mg/Ml. solutions were then incubated at 37ºC and sample was taken at predetermined time points and checked by LC-MS technique.

Cells were incubated at 37 °C in a humidified atmosphere of 5% CO_2 and kept in 258 logarithmic phase of growth throughout all experiments. BxPC-3, Capan-1, Panc-1 PSC and PDAC pancreatic cancer cell line.

Results:

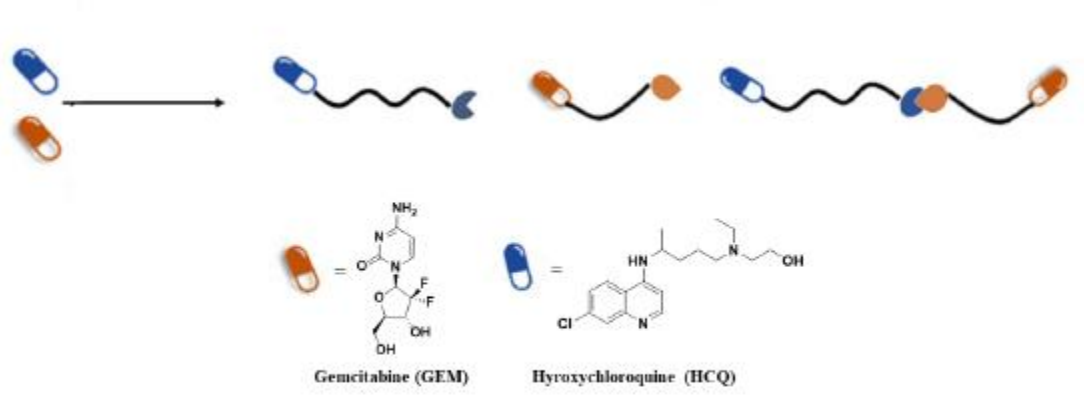
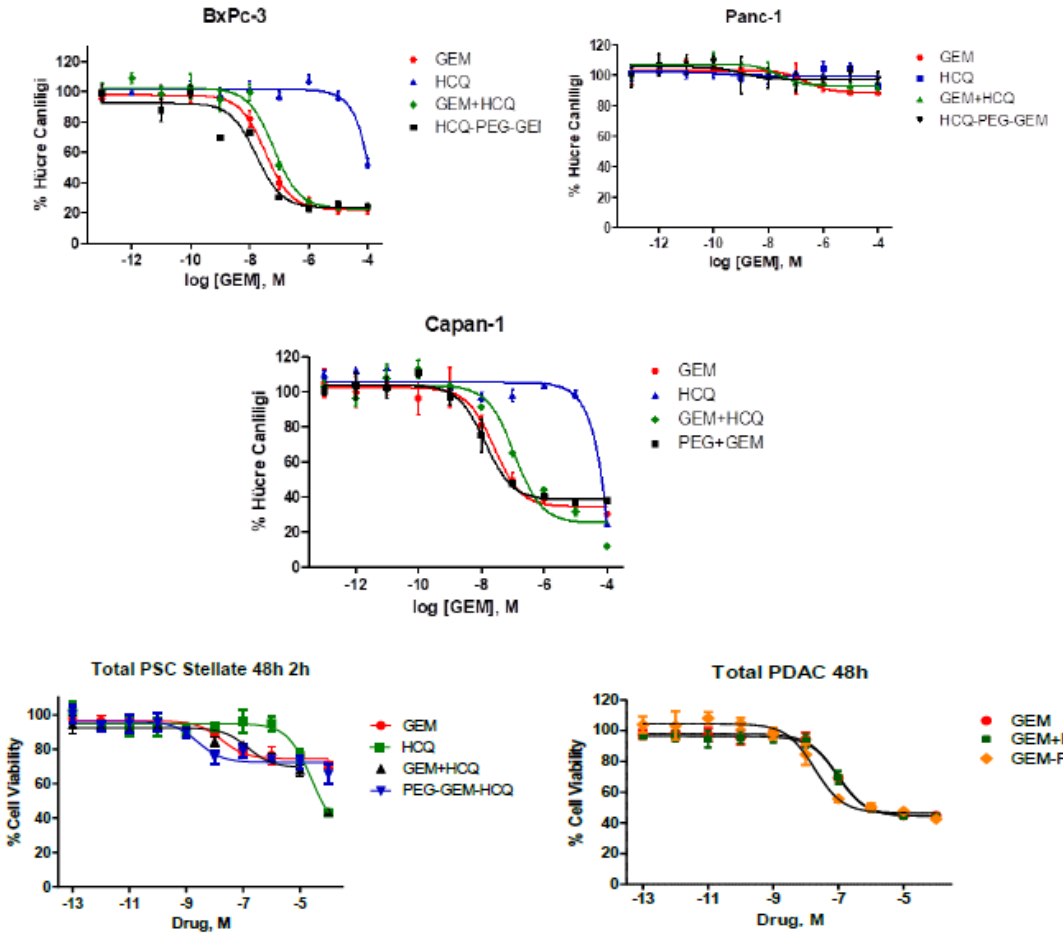


Figure1: HCQ-PEH-GEM synthesis.



Constructs	EC_{50} values (M)				
	BxPC-3	Capan-1	Panc-1	PSC	PDC
GEM	3.39×10^{-8}	2.51×10^{-8}	2.21×10^{-7}	1.75×10^{-8}	6.21×10^{-8}
HCQ	$>10^{-5}$	$>10^{-4}$	$>10^{-4}$	2.63×10^{-6}	
GEM+HCQ	6.63×10^{-8}	1.16×10^{-7}	2.36×10^{-8}	1.48×10^{-7}	1.007×10^{-7}
HCQ-PEG-GEM	1.68×10^{-8}	1.3×10^{-8}	$>10^{-4}$	3.53×10^{-9}	1.85×10^{-8}

Conclusion: The Dual Prodrug was obtained via multistep organic synthesis. Its cytotoxicity was examined on different Pancreatic cancer cell lines. The drugs release was studied into buffers at 5.4 and 7,4 pH values.

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Acknowledgments:

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Chemically Engineered Iron Oxide Nanocrystals for Transport of Biomolecules Across Biological Barriers

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Chemical processing of functional ceramics has played a key role in converging disciplines, which is especially true for their bridge-building role in integrating the concepts of inorganic materials synthesis with biomedical applications. Out of a vast variety of metal and metal oxide nanoparticles that have been developed for medicinal purposes, iron oxides are one of a few materials that made it through clinical trials. Due to their high biocompatibility, stability and the abundance of iron in our environment, which results in low costs of iron-based materials, diverse iron oxide nanoparticles (IONPs) have been prepared for biomedical applications. In our workgroup, ɣ-Fe_2O_3, α-Fe_2O_3 and Fe_3O_4 based IONPs have been synthesized using a broad range of well-established synthetic procedures. By changing the reaction conditions and applying suitable surface ligands, the morphology (spherical, cube-shaped, ellipsoidal), surface charge and dispersibility of IONPs could be tuned according to the desired application allowing for a reproducible fabrication of optimized and highly efficient vectors. Controlled surface vectorization with biomolecules led to the formation of cancer targeting platforms, while the employment of the highly selective click chemistry enabled the magnetic separation of proteins out of a proteome mixture. Moreover, as-prepared particles could be used for drug delivery applications, either through covalent attachment of a drug to the particle surface or by using the IONPs as templates to prepare hollow drug containers. This talk will present how chemically grown nanoparticles can be transformed into bio-vectors for magnetic resonance imaging (MRI) and drug delivery applications.

Multivalent carbosilane glycodendrimers designed for bioapplications

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An interplay of multiple interactions of carbohydrates and proteins promotes critical events in biological processes. The synthetic control over the dendritic structure in terms of size, shape, multivalent presentation of ligands, and drug encapsulation predetermines dendrimers as optimal ”glycocarriers” in nanomedicine[1]. In drug delivery, dendritic compounds served as nanocarriers for a variety of drugs with the capacity to improve their solubility, bioavailability, and to ease the undesired toxic effect[2].

Recently, we developed a robust and flexible synthetic route to conjugate carbohydrates to the periphery of carbosilane dendrimers (CS-DDMs). To boost multivalent presentation, we tailored CS scaffolds with a derivative of 4-hydroxy isophthalic acid to double the amount of peripheral reactive sites. Therefore, we synthesized three series of 1st – 3rd generation CS glyco-DDMs bearing gluco- and galacto- ligands conjugated to the molecule directly, or via a short oligo ethylene glycol linker to enhance biocompatibility and hydrosolubility of the compounds. To survey the biochemical properties, we evaluated the cytotoxicity of the glyco-DDMs against both non-cancer (BJ) and cancer (A2780 and MCF7) cells revealing their exceptional biocompatibility[3].

The anthracycline doxorubicin (DOX), one of the most powerful chemotherapeutics, still raises concerns regarding its toxicity towards non-targeted tissues. In our study, we encapsulated

molecules of DOX into the glyco-DDMs to reveal the potential of the conjugates in drug delivery. The resulting glyco-DDM/DOX complexes showed promising anticancer activity, especially against A2780 cancer cell line. Considering negligible hematotoxicity and favourable drug release kinetics, we may consider glyco-DDM/DOX complexes as promising drug delivery systems in cancer therapy.

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Molecular Dynamics&NMR of Peptide Dendrimers with Dipeptide Spacers

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Background: Dendrimers contain central core, branched units and terminal groups. Lysine dendrimers were first peptide dendrimers consisting of lysine aminoacid residues. Simplest lysine dendrimers have lysine core, lysine branched repeating unit and lysine terminal groups. In present work we studied properties of lysine dendrimers with the dipeptide spacers (2Gly or 2Lys or 2Arg) inserted between each pair of neighboring branched lysine residues. Such dendrimers have the same core, same branched backbone and the same terminal groups. The only difference between them is the side groups of their spacers. Thus the dendrimers have repeating units Lys2Gly, Lys2Arg, Lys2Lys. 2Gly spacers have no charge while 2Lys and 2Arg spacers have charge equal +2. It means that Lys2Gly has only surface charge while other two dendrimers have charge distributed through volume of dendrimer.

Experimental: It is well known that dendrimers usually have spherical shape, precise size and many terminal groups capable for functionalization (PCCP, 2016, 18, 24307; Polymer, 2017, 125, 292; Pharmaceutics, 2018, 10, 129; Polymer, 2018, 146, 256; Macromolecules, 2020, 53, 7298). Lysine dendrimers (Dendrimers in Biomedical Applications, RSC, 2013, 99-114; Polym.Sci, Ser. C., 2013, 55, 154; PCCP 2015, 17, 3214; Langmuir, 2018, 34, 1613) and peptide dendrimers studied here (Int. J. Mol. Sci. 2020, 21, 9749; Polymers, 2020,12(8), 1657) were recently tested as nanocarriers for gene delivery (Bioorg.Chem., 2020, 95, 103504; Int. J. Mol. Sci. 2020, 21, 3138;).

Results: Here we studied temperature dependences of equilibrium characteristics and orientational relaxation times of main chain, side chain and terminal CH2-N groups between T=283K and 343 K. We obtained that size, shape, radial density profile and radial charge distribution of these dendrimers almost do not depend on temperature. Temperature dependences of relaxation times and spin-lattice relaxation time T_{1H} of CH_2-N groups of all dendrimers were calculated from NMR experiments and MD simulation.

Conclusion:. It was shown that results of MD simulation are very close to experimental results obtained for these dendrimers by NMR (Sci. Rep. 2018, 8, 8916; Molecules, 24, 2019, 2481; RSC Adv. 2019, 9, 18018;).

Acknowledgments: This work was supported by RSCF grant 19-13-00087. All computer simulations were carried out using computer facilities of SPbSU and MSU Supercomputer Centers.

Hybrid of Ag@SiO_2 nanoparticles *via* a modified sol-gel method for theranostic applications in cancer

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Recently, there has been ongoing research in the field of nanotechnology and nanomedicine aiming at developing multifunctional biomaterials using noble metals.

In order to improve the biocompatibility of silver nanoparticles different approaches are employed. The formation of silica coating known as sol-gel method has several advantages such as numerous opportunities on the surface modification of the nanoparticles that can lead to the design and synthesis of multifunctional nanoparticles.

Silica is considered biodegradable and offers a variety of functional groups for the further modification of the nanosystems with drugs and/or targeting agents. Although the sol gel method is thoroughly studied and there are numerous examples in bibliography, there are some difficulties in the case of silver. The selection of the base catalyst of the reaction as we observed can have a high impact on the properties of the synthesized nanoparticles.

The hybrid nanoparticles were characterized structurally for the determination of their size and shape, while the modification of their surface with amine groups was monitored and quantified. Their biological evaluation was conducted with different *in vitro* methods to assess their cytotoxicity to different cell lines and red blood cells and observe their cellular uptake *via* fluorescence microscopy, which highlighted their anticancer properties and vivid cell imaging potential.

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RNA G-quadruplexes in pre-miRNAs: A new way in the target of nucleolin

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G-quadruplexes (G4s) came into the trend of research in the last few years due to their intrinsic features and strengths over DNA G4s. RNA G4s have been characterized in non-coding RNAs, such as primary microRNA precursors, precursor microRNAs and microRNAs, indicating the potential of these motifs to control and impact miRNA biogenesis and targeting [1,2]. Moreover, considering recent evidence, some proteins modulate RNA G4 function and stability. Among G4 interacting proteins, nucleolin (NCL) is the protein most often reported for its functions upon G4 recognition. It is generally believed that NCL helps the folding of G4 structures. Furthermore, cell surface NCL is the target of proliferation inhibitor molecules in cancer cells. The overexpression of NCL and increased localization at the cell membrane was shown in several cancer cell lines. Bearing this in mind, the interaction of NCL with G4s has been closely related to disease namely, cancer, and could be used to develop novel diagnostic and therapeutic strategies.

Herein, we have studied three RNA G4 sequences present in pre-miRNA-149, -92b and -let7e. The formation and stabilization of each RNA G4 sequence were assessed by circular

dichroism (CD) and nuclear magnetic resonance (NMR). Thereafter, in order to investigate the ability of ligands to stabilize or destabilize the RNA G4 structures, we employed CD- and FRET- melting experiments. The formation of the supramolecular complexes RNA G4/ligand, RNA G4/NCL and pre-RNA G4/ligand/NCL complexes were checked by polyacrylamide gel electrophoresis (PAGE). The binding of RNA G4 to NCL in a cellular context was performed by confocal microscopy.

Our results suggested the formation of RNA G4s by the guanine-rich sequences of pre-miRNA-149, 92b and let7e. The stability of the RNA G4 sequences was achieved by adding G4 ligands as demonstrated by CD- and FRET-melting experiments. The RNA G4 found in pre-miRNA-149 showed remarkable ability as a supramolecular carrier of a G4 ligand to cancer cells.

Overall, this study could pave the way for future approaches that target nucleolin for therapeutic purposes.

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Photoactivatable nanoCRISPR/Cas9 system

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Background: The design of nucleic acids constructions containing photosensitive residues or linkers permits to create spatiotemporal regulated system. Photocaged oligonucleotides have been used for photoactivation of CRISPR/Cas9 gene editing systems [1,2]. Here we proposed to immobilize crRNA through the photocleavable oligodeoxyribonucleotide (PC-DNA) on carbon nanoparticles (CNP) surface for the transfection of gene editing system components into the cell with subsequent activation of nanoCRISPR/Cas9 by UV-irradiation.

Experimental: PC-DNA complementary to crRNA with two or three photocleavable linkers inside the chain and with pyrene residue at 3'-terminus have been synthesized. The stability of duplexes of pyrene modified PC-DNA with crRNA were studied by gel-shift assay and thermal denaturation method. The isotherms of crRNA/PC-DNA duplexes immobilization on the surfaces of CNP were obtained using methods of pyrene fluorescence quenching [3] (Figure). The release of fluorescent crRNA upon UV-irradiation was examined by PAGE analysis. Cell penetration crRNA/PC-DNA/CNP complex was investigated by cytofluorometry. The immobilized crRNA were used *in vitro* as component of nanoCRISPR/Cas9 system.

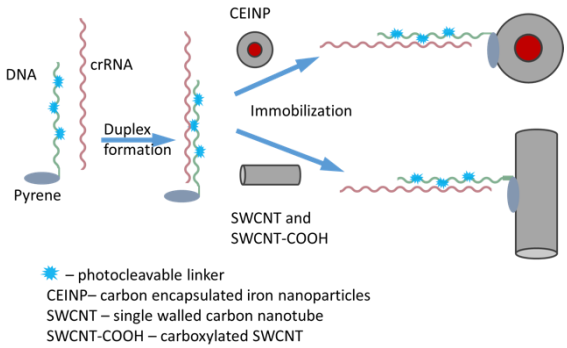

Figure. Immobilization of crRNA/PC-DNA duplex on the CNP surface.

Results: The possibility of PC-DNA photodegradation and crRNA released from CNP surface as well as nanoCRISPR/Cas9 system activation by UV-irradiation was demonstrated.

Conclusion: The proposed approach for the design of CNP-immobilized photoactivatable crRNA for cell transfection and UV-activation of CRISPR/Cas9 system is prospective for spatiotemporal gene editing.

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Boron nitride nanoparticles as compounds dedicated to boron neutron capture therapy

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Background: Boron neutron capture therapy (BNCT) is classified as a targeted anti-cancer radiotherapy based on boron delivery to tumor cells and irradiation of the affected area with a neutron beam [1]. One of the leading challenges for the development of BNCT is the search for new boron-rich compounds that will allow to achieve the required concentration of ^{10}B isotope in cancer cells. Boron nitride nanoparticles, due to their high boron content, are becoming an object of interest for application in BNCT [2]. The problem that needs to be solved is the way of their delivery to the cancer cells. We propose an original approach to use macrophages/monocytes for boron delivery to the tumor microenvironment. These professional phagocytic cells are distributed widely in the body tissues and are strongly associated with cancer tissues [3].

Experimental: The newly synthesized boron nitride nanoparticles have been characterized in terms of shape and size using transmission electron microscopy and dynamic light scattering measurement. In order to evaluate the toxicity of tested compounds, the MTT cell viability assay and Annexin V/propidium iodide apoptosis assay were conducted on RAW 264.7 monocyte/macrophage-like cells. Additionally, to determine cytokines production by these cells, an ELISA test (enzyme-linked immunosorbent assay) was performed.

Results: We selected 2 newly synthesized boron nitride preparations (BN-1, BN-6) with a size not exceeding 250 nm for biological examination. Our results demonstrated that RAW 264.7 cells tolerated boron nitride in a concentration up to 100 μg/ml, above this concentration tested compounds appears to be toxic. We also revealed the dose-dependent production of TNF-α by macrophages.

Conclusion: Boron nitride nanoparticles transported in cells of the immune system could become a potential candidate for boron neutron capture therapy. Additionally, macrophages upon stimulation by the tested compounds can modulate the tumor microenvironment through the production of cytokines.

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Tyrosine kinase inhibitors (TKIs)-nanocarriers for precision medicine treatment of acute leukemias

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Nano-based drug delivery systems are among the newest and most promising tools in nanomedicine [1]. By interfacing nanoparticles with various coatings of particular physical-chemical functionality (e.g. amphiphilicity, pH or thermal-sensitivity) one can develop vectors for loading and controlled delivery of problematic cargo molecules such as Tyrosine Kinase Inhibitors, a class of hydrophobic drugs with high potential in personalized cancer treatment [2].

This work is focused on the development of nanoparticle-carriers that can serve as efficient vehicles for the selective delivery of tumor inhibitor drugs against several lineages of leukemias. To fabricate the nanocompounds we loaded Midostaurin and Dasatinib drugs onto gold nanoparticles of various morphologies (spherical, ellipsoidal, hollow) and hence of modulated capacity for particle retention, clearance and tunable optical properties [3]. Further on, we conjugated the drug-loaded particles with a series of stimuli-responsive polymers (Pluronic, poly-lactic-co-glycolic acid, poly-2 dimethylamino-ethyl methacrylate, and poly-

vinyl-pyrrolidone) with role in chemical stabilization and biocompatibilization. The selected drug molecules act upon specific biological structures in key points in the cell cycle, particularly the fms related tyrosine kinase 3 (FLT3) in acute myeloid leukemia, and the BCR-ABL tyrosine kinase in Philadelphia(+) leukemias respectively, by inhibiting their signaling capacity. The controlled release of the drugs and the cellular pharmacokinetics of the nanocomplexes were investigated in simulated biological media and on MV4-11 acute myeloid leukemia and SUP-B15 acute lymphoblastic leukemia cell lines.

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Docetaxel loaded magnetic nanoparticles based on functionalized chitosan with potential theranostic applications: synthesis, characterization and *in vitro* studies

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Background: Cancer is the leading cause of worldwide death for centuries, accounting millions of deaths yearly. Current therapies among which chemotherapy, radiotherapy and surgery remain still limited, mainly due to their lack of selectivity, multidrug resistance and severe toxic effects.[1] Nanotechnology could help overcome these limitations, sustained by the promising results dedicated to the development of polymer based magnetic nanoparticles for theranostic applications.[2] Functionalized superparamagnetic iron oxide nanoparticles (SPIONs) are an important class of drug delivery carriers due to their remarkable features: controlled and sustained drug release, targeted therapy by magnetic steering and imaging capabilities.[3]

Experimental: The present study proposes the development of Docetaxel loaded magnetic nanoparticles based on functionalized chitosan in three steps: *i)* hydrophobic iron oxide nanoparticles through partial oxidation reaction and coverage with sodium oleate under specific conditions, *ii)* biocompatible surface coating based on chitosan and biotin, that ensures physiological stabilization medium, multifunctionality and tumour cell recognition layer *via* carbodiimide chemistry, *iii)* synthesis of loaded drug magnetic functionalized chitosan

nanoparticles by self-assembly method followed by ionic gelation with sodium tripolyphosphate.

Results*:* The physicochemical properties of Docetaxel loaded magnetic functionalized chitosan nanoparticles were evaluated in terms of size, surface charge, composition and morphology. Dynamic light scattering data indicated adequate size distribution and a negative Zeta potential. FT-IR spectroscopy combined with thermogravimetric analysis confirmed that the structure of the nanoplatforms contains: a magnetic core, a polymeric shell and Docetaxel. Preliminary *in vitro* investigations demonstrated the nanoparticles ability to deliver Docetaxel in simulated biological fluids and degradation potential in the same medium. Cell viability assays carried out on MCF-7 and MDA-MB-231 cell lines using MTT test confirmed the compatibility for the drug free nanoparticles and a decrease for the loaded nanoparticles.

Conclusion*:* Overall, the physicochemical and preliminary in vitro results showed the potential of Docetaxel loaded magnetic nanoparticles based on functionalized chitosan to be used as theranostic systems. Further *in vitro* and *in vivo* tests are still needed to tailor the appropriate nanosystem for the envisioned application.

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