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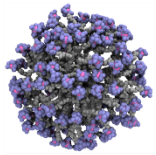

COST ACTION CA 17140
NANO2CLINIC
CANCER NANOMEDICINE - FROM THE BENCH TO THE BEDSIDE

COST ACTION CA17140
NANO2CLINIC

1st STSM VIRTUAL CONFERENCE

BOOK OF ABSTRACTS

MARCH 16, 2022

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CONTENT

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PROGRAMME

❖ Opening (09:00)

Presenters: Sabrina Pricl (Action Chair); Maria Francesca Ottaviani (Grant Awarding Coordinator)

❖ Guidelines for clinical trials and regulatory aspects

Session chair: Darío Manzanares Sandoval

09:15 – 09:45 ***Plenary lecture***
Nissim Garti, Sharon Garti- Levi and Rotem Edri
"Novel in-situ formation of thin film embedded with nano-domains coroneted by drug molecules for controlled transdermal release of drugs"

09:45 – 10:00 ***Short oral***
Virginia Cazzagon, C. Fito
"Development of an occupational risk assessment for nanobiomaterials used in Advanced Therapy Medicinal Product for cancer treatment"

❖ Manufacturing nanodrugs Part I

Session chair: Elham Poonaki

10:00 – 10:15 ***Short oral***
M. Hovorková, D. Goyard, J. Červený, V. Křen, O. Renaudet, P. Bojarová.
"Advanced High-Affinity Glycocluster Ligands of Galectins"

10:15 – 10:30 ***Short oral***
Valeria Arkhipova, Nadezhda Knauer, Ekaterina Pashkina, Alina Aktanova, Javier Sánchez-Nieves, Francisco Javier de la Mata, Rafael Gómez, Evgeny Apartsin
"Synthesis of amphiphilic carbosilane dendrons for cancer nanomedicine"

10:30 – 10:45 ***Short oral***
Antonín Edr, E. Apartsin, J. Kalasová, J. Malý and T. Strašák
"Carbosilane Dendritic Amphiphiles for Cancer Nanomedicine Drug Delivery"

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10:45 – 11:00 ***Short oral***
Joana Figueiredo, Israel Carreira-Barral, Roberto Quesada, Jean-Louis Mergny, Carla Cruz
"Synthesis and evaluation of phenanthroline-based derivatives as human pre-MIR150 G-quadruplex binders for lung cancer therapy"

11:00 – 11:15 ***Short oral***
André Miranda, Roi Lopez-Blanco, Eduardo Fernandez-Megia, Carla Cruz
"Development of aptadendrimers for prostate cancer therapy"

11:15 – 11:30 ***Short oral***
Dina Maciel, Dominika Wróbel, João Rodrigues, Tomáš Strašák and Monika Müllerová
"Carbosilane glycodendrimers for anticancer drug delivery"

❖ Manufacturing nanodrugs Part II

Session chair: Natalia Sanz del Olmo

11:30 – 11:45 ***Short oral***
Kinga Skrzyniarz, Javier Sanchez-Nieves, Andrea Barrios-Gumiel, Sara Quintana, Karol Ciepluch
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Elham Poonaki , Ann-Christin Nickel , Mehdi Shafiee Ardestani, Ali Gorji, Sven G. Meuth, E. Apartsin, Christoph Janiak, U Kahlert
"CD-133 functionalized Gold nanoparticles loaded CB839 targeted drug delivery suppressed Glioblastoma stem cells"

❖ Physico-chemical characterization of nanodrugs Part I

Session chair: Dilara Buse Durdabak

12:00 – 12:15 ***Short oral***
Viktor Andonovic and Aleksandar Dimitrov
"Algorithm based on XRD data for determining the number and distribution of layers of graphene as drug delivery nanocarrier"

12:15 – 12:30 ***Short oral***
Beti Andonovic, A. T. Dimitrov and J. Djordjevic
"Structural Characterization and determination of topological indices of Carbon Nanomaterials:

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Graphene and CNTs"

12:30 – 12:45 ***Short oral***
Marina Kovacevic, I. Balaz, D. Marson , E. Laurini, B. Jovic
"Structure of mixed-monolayer gold nanoparticles: Molecular Dynamics study"

12:45 – 13:00 ***Short oral***
Maria José Silveira, María de La Fuente, Maria José Oliveira, Bruno Sarmento
"Development of novel targeted immunomodulatory nanoparticles for colorectal cancer treatment"

13:00 – 13:15 ***Short oral***
Aleksandar T. Dimitrov, Beti Andonovic, Zeljko Kamberovic
"Characterization of Carbon nanostructures"

13:15 – 13:30 ***Short oral***
Jose Antonio Laz-Ruiz, Maria Victoria Cano-Cortes, Juan Jose Diaz-Mochon, Tore-Geir Iversen and Rosario Maria Sanchez-Martin
"Preclinical characterization of biocompatible multifunctional nanodevices for cancer nanomedicine"

❖ Physico-chemical characterization of nanodrugs Part II

Session chair: Jose Antonio Laz-Ruiz

13:30 – 13:45 ***Short oral***
Lucija Božičević, A. Iskrzynska, M. Loparić, I. Vinković Vrček
"Nanomechanical tool for characterization of breast cancer cells"

13:45 – 14:00 ***Short oral***
Michal Gorzkiewicz, D. Appelhans, S. Boye, A. Lederer, B. Voit, B. Klajnert-Maculewicz
"Complexation properties of glycodendrimers towards nucleotides"

14:00 – 14:15 ***Short oral***
Tamara Rodríguez-Prieto, Alberto Fattori, Claudimar Camejo, F. Javier de la Mata, Jesús Cano, M. Francesca Ottaviani, Rafael Gómez
"Study of imidazolium-terminated carbosilane dendritic systems by EPR"

14:15 – 14:30 ***Short oral***
Barbara Pem, V. Vrček, A. Adamatzky and I. Vinković Vrček
"Computational approach to the study of nano-bio interface"

14:30 – 14:45 ***Short oral***

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Rafael Ramírez-Jiménez, R. Barbir, K. Ilić, E. Galic, B. Pem, I.Pavičić, R. Martín-Rapún, J. Martínez de la Fuente and I. Vinković Vrček
"Drug delivery of doxorubicin with gold nanoparticles"

14:45 – 15:00 ***Short oral***
Esperanza Padín González, Elena Navarro Palomares, Marco Monopoli, Mónica López Fanarraga
"Customizing the identity of nanomaterials to enhance the targeting"

❖ Physico-chemical characterization of nanodrugs Part III

Session chair: Darío Manzanares Sandoval

15:00 – 15:15 ***Short oral***
Natalia Sanz del Olmo , C. de la Torre, V. Ceña and M. Malkoch.
"*Cationic amino functional bis-MPA based linear-dendritic block copolymers as non-viral vectors of siRNA*"

15:15 – 15:30 ***Short oral***
Rinea Barbir, R. Ramírez Jiménez, R. Martín-Rapún, V. Strasser, D. Domazet Jurašin, S. Dabelić, J. M. de la Fuente, and I. Vinković Vrček
"Interaction of Differently Sized, Shaped, and Functionalized Silver and Gold Nanoparticles with Glycosylated versus Nonglycosylated Transferrin

15:30 – 15:45 ***Short oral***
Dilara Buse Durdabak, S. Dogan, M. Eser, K. Başak, C. O'Sullivan, B. Guvenc Tuna
"Development of Lateral Flow Assay for Detection of DOX-Resistant Breast Cancer Biomarker"

15:45 – 16:00 ***Short oral***
Dominika Wróbel, A. Fattori, M. Müllerová, T. Strašák, J. Malý, M. F. Ottaviani
"Glucose-decorated Carbosilane dendrimers interacting with a model cell membrane, studied by means of the spin-probe EPR technique"

❖ Preclinical studies of nanodrugs Part I

Session chair: Natalia Sanz del Olmo

16:00 – 16:15 ***Short oral***
Dominika Wróbel, A. Janaszewska, M. Müllerová, T. Strašák, J. Malý and B. Klajnert-Maculewicz

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"Anti-inflammatory properties of carbosilane dendrimers"

16:15 – 16:30 *Short oral*
Monika Sramkova and I. V. Vrcek.
"*Advanced in vitro models for (nano)toxicity determination*"

16:30 – 16:45 *Short oral*
Kristina Jakic, M. Sramkova, A. Babelova, S. Spring, M. Müller, T. Knoll, Y. Kohl and A. Gabelova.
"*A microfluidic model of the kidney – a platform for the determination of renal toxicity*"

16:45 – 17:00 *Short oral*
Emerik Galić, Y Richaud, I. Fernández Carasa, I. Vinković Vrček and A. Raya.
"*Stem cells as in vitro model for testing nanodrug delivery properties of selenium nanoparticles*"

17:00 – 17:15 *Short oral*
Filipe Olim, A. R. Neves, V. Ceña and H. Tomás.
"*Fucoidan/dendrimer nanoparticles for glioblastoma treatment: in vitro siRNA delivery studies*"

17:15 – 17:30 *Short oral*
Nadezhda Knauer, V. Arkhipova, R. Gómez, J. Sánchez-Nieves, E. Pashkina, V. Kozlov, E. Apartsin and U. Kahlert.
"*Dendrimers for microRNA delivery into human glioblastoma stem-like cells*"

❖ Preclinical studies of nanodrugs Part II

Session chair: Natalia Fernández Bertólez

17:30 – 17:45 *Short oral*
Monika Marcinkowska, A. Janaszewska and B. Klajnert-Maculewicz.
"*Influence of PAMAM-taxanes-monoclonal antibody conjugates on the breast cancer cells*"

17:45 – 18:00 *Short oral*
Danai Prokopiou, V. Kusigerski, S. Vranjes-Duric and E. K. Efthimiadou.
"*Iron oxide magnetic nanoparticles modified with biomolecules for theranostic applications*"

18:00 – 18:15 *Short oral*
Anna Janaszewska, D. Wróbel, M. Liegertová, J. Maly, M. Marcinkowska and B. Klajnert-Maculewicz.
"*Application of auto–fluorescent nanoparticles as a new bioimaging agents in vivo*"

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18:15 – 18:30 ***Short oral***
M. Giannakou, N. Evripidou, A. Antoniou, and Christakis Damianou.
"*Manual positioning device for FUS induced BBB disruption in mice*"

18:30 – 18:45 ***Short oral***
Jéssica Lopes-Nunes, J. Lifante, Y. Shen, E. C. Ximendes, M. C. Iglesias-de la Cruz, D. Jaque, C. Cruz
"ICG-tagged aptamer as drug delivery system for malignant melanoma"

18:45 – 19:00 ***Short oral***
Atida Selmani, I. Vidaković, C. J. Hill, R. Prassl and I. Vinković Vrček
"Stability of selenium nanoparticles as novel anticancer delivery vehicle in relevant biological media"

❖ Closure (19:00)

Presenters: Sabrina Pricl, Maria Francesca Ottaviani

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PLENARY LECTURE

Novel in-situ formation of thin film embedded with nano-domains coroneted by drug molecules for controlled transdermal release of drugs"

Nissim Garti[1], Sharon Garti- Levi and Rotem Edri[2]

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The present was done in collaboration with LDS, private biotech company established by Prof Garti and Dr Sharon Garti-Levi

In the last 8 years we have developed, after exploring several thermodynamic and geometrical models a novel liquid nanodomains (15-25nm) different from classical microemulsions in size and shape. The nanodomains are capable of embedding large amounts of solubilizates (bioactives) and deliver them across membranes "On Demand" and in controlled pattern.

Recently, we developed novel technology formation of adhesive very thin and transparent film, formed in-situ, upon applying the liquid nano domains coroneted with the bio actives and other components, on skins and other surfaces.

We will bring some models and data on the formation of the thin films and on possible applications.

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SHORT ORAL PRESENTATIONS

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Structural Characterization and determination of topological indices of Carbon Nanomaterials: Graphene and CNTs

B. Andonovic[1], A. T. Dimitrov[1] and J. Djordjevic[2]

[1]Faculty of Technology and Metallurgy, St. Cyril and Methodius University, Skopje, N. Macedonia
[2]University of Nis, Nis, Serbia

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The interest for the intensive studies and methods of structural characterization of multiwall carbon nanotubes (MWCNTs) to date has resulted in many valuable contributions and an amazingly wide application area. This research offers an approach that combines several techniques. It is the first direct application of the graph theory upon nanotubical structures obtained by electrolysis in molten salts using non-stationary current regimes. The spectroscopic data enables studying the diameters, as well as performing an (*n*,*m*) assignment of nanotube samples (see Figure 1). Using the graph representation and the chirality of the studied samples, different distance based topological indices (Wiener, Balaban, Sum-Balaban, and Gutman indices) have been evaluated in order to enable further prediction of index-related properties of the molecules. [1,2,3]

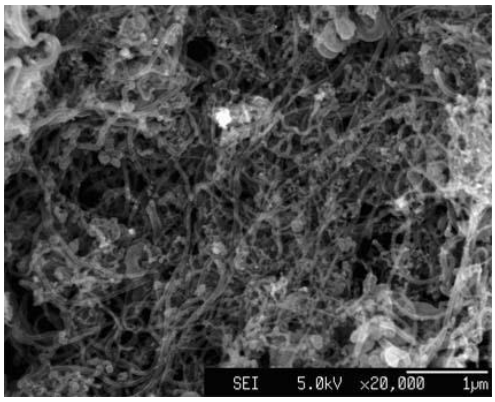

Figure 1. SEM image of CNTs obtained by electrolysis in molten salts.

References

1. Schwandt C., Dimitrov A. T.; Fray D. J. High-yield synthesis of multi-walled carbon nanotubes from graphite by molten salt electrolysis. Carbon. 2012; 50:1311–1315.
2. Andonovic B., Ademi A., Grozdanov A., Paunović P., Dimitrov A. T. Enhanced model for determining the number of graphene layers and their distribution from X-ray diffraction data. Beilstein J. Nanotechnol. 2015; 6: 2113-2122
3. Andova V., Knor M., Škrekovski R. Distances on nanotubical structures. R. J. Math. Chem. 2016, 54: 1575-1584.

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Algorithm based on XRD data for determining the number and distribution of layers of graphene as drug delivery nanocarrier

Viktor Andonovic[1] and Aleksandar Dimitrov[2]

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We developed an improved model for determining the number of graphene layers and their distribution. An analysis was performed upon graphene samples produced by two electrochemical procedures, namely, electrolysis in molten salts and electrolysis in aqueous electrolyte, both using a nonstationary current regime. The model is enhanced by a partitioning of the corresponding 2θ interval, which results in significant improvement in the accuracy of the results. The obtained model curves exhibit excellent fitting to the XRD intensities curves of the studied graphene samples. The parameters of the model enable the calculation of the coverage of the *j*-layer graphene region of the graphene samples, and subsequently the number of graphene layers. The results of the thorough analysis correspond with the calculated number of graphene layers from Raman spectra C-peak position values and indicate that the graphene samples studied are few-layered. Having the evidence that graphene sheets used in research usually have asymmetrical 002 XRD peaks, the model is intended for general use, since such peaks are inconvenient to be tackled by the simple models in the literature. The implementation of the algorithm and the analysis within the study were performed in Python.

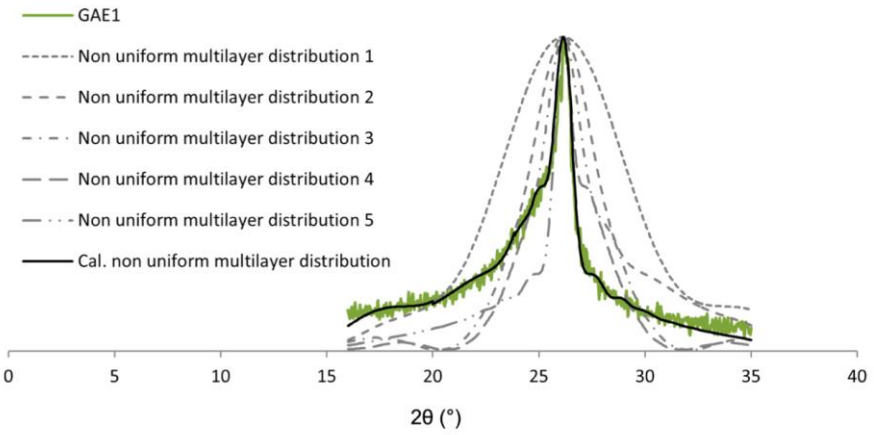

Figure 1. Nonuniform multilayer distribution curve for Graphene Sample Produced by Electrolysis in Aqueous Electrolyte (GAE)

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Synthesis of amphiphilic carbosilane dendrons for cancer nanomedicine

Valeria Arkhipova [1,2], Nadezhda Knauer [3], Ekaterina Pashkina [3], Alina Aktanova [3], Javier Sánchez-Nieves [4,5], Francisco Javier de la Mata [4,5,6], Rafael Gómez [4,5,6], Evgeny Apartsin [1,2,7]

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[4] *Departamento de Química Orgánica y Química Inorgánica, UAH-IQAR, Universidad de Alcalá, 28805 Alcalá de Henares, Spain*
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[6] *Instituto Ramón y Cajal de Investigación Sanitaria, IRYCIS, 28034 Madrid, Spain*
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Dendritic molecules, dendrimers and dendrons, are versatile platforms to design supramolecular nanoconstructions for robust drug delivery. Dendron molecules are stable, biocompatible and biodegradable, and their synthesis is easily controlled at every stage. Therefore, drug delivery systems based on this type of polymer can expect rapid approval by regulating entities. Systematic studies conducted using amphiphilic dendrons revealed the effect of both the structure of the hydrophobic part and the dendron generation as amphiphilic part on their assembly into supramolecular constructions of given topology: micelles, unilamellar or multilamellar vesicles. It has been proven that low molecular therapeutic compounds can be successfully encapsulated inside such nanoparticles. Replacement of various fragments of a hybrid molecule with analogs can lead to a radical improvement in the properties of nanoparticles. Approach to the block synthesis of hybrid amphiphilic triazine-carbosilane dendrons with linkers of different nature (piperazine and aminohexanoate) and cationic groups on the surface was developed. The key difference between two linkers is that the piperazine group is capable of ionization under physiological conditions. As a branched fragment we chose carbosilane molecules generations 2 and 3 ($-Si(CH_3)<$ as a branching unit and the linkers between the units are $-(CH_2)_3-$ fragments). The surface of the dendrimers was functionalized with tertiary amino groups, which provides the solubility of molecules in water. Synthesized molecules, due to the balance of hydrophobic and hydrophilic parts, self-organize into soft nanoparticles of micellar or vesicular topology, and can be loaded with anti-tumor therapeutics, showing efficient accumulation in leukemia cells and high cytotoxic activity [1].

References

1. Apartsin, E., Knauer, N., Arkhipova, V., Pashkina, E., Aktanova, A., Poletaeva, J., Sánchez-Nieves, J., de la Mata, F. J., & Gómez, R. (2020). pH-sensitive dendrimersomes of hybrid triazine-carbosilane dendritic amphiphiles-smart vehicles for drug delivery. Nanomaterials, 10(10), 1899.

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Interaction of Differently Sized, Shaped, and Functionalized Silver and Gold Nanoparticles with Glycosylated versus Nonglycosylated Transferrin

R. Barbir[1], R. Ramírez Jiménez[2], R. Martín-Rapún[2], V. Strasser[3], D. Domazet Jurašin[3], S. Dabelić[4], J. M. de la Fuente[2], and I. Vinković Vrček[1]

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[2]*Instituto de Nanociencia y Materiales de Aragón (INMA), Universidad de Zaragoza – CSIC and CIBER-BBN, Zaragoza, Spain*
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Possible application of metal nanoparticles (NPs) in medicine has been challenged by numerous factors, especially regarding their biocompatibility, efficacy, and safety. Exposure of NPs to a biological medium results in their direct interaction with biological macromolecules and leads to the formation of a dynamic biomolecular layer known as the biomolecular corona [1,2]. Despite numerous published data on nano-biointeractions, the role of protein glycosylation in the formation, characteristics, and fate of such nano-biocomplexes has been almost completely neglected, although most serum proteins are glycosylated [3]. This study aimed to systematically investigate the differences in interaction of metallic NPs with glycosylated vs nonglycosylated transferrin. To reach this aim, we compared interaction mechanisms between differently sized, shaped, and surface-functionalized silver and gold nanoparticles to commercially available human transferrin (TRF), a glycosylated protein, and to its nonglycosylated recombinant form (ngTRF). Bovine serum albumin (BSA) was also included in the study for comparative purposes. After performing NPs characterization fluorescence quenching and circular dichroism methods were used to evaluate protein binding constants on the nanosurface and conformational changes after the protein–NP interactions, respectively. Competitive binding of TRF, ngTRF, and BSA to the surface of different NPs was evaluated by separating them after extraction from protein corona by gel electrophoresis following quantification with a commercial protein assay. It was found that the strength of protein binding to NPs, changes in secondary protein structure and composition of protein corona depend not only on the physicochemical properties of NPs, but also on the presence of glycans on proteins. The obtained results emphasize the importance of protein glycosylation for nano-bio interactions, which contributes to understanding the effect of NPs on biological systems and can be used in the development of new diagnostic, prognostic and therapeutic nanotools.

References

1. Capjak I, Goreta SŠ, Jurašin DD et al. How protein coronas determine the fate of engineered nanoparticles in biological environment. Arch Ind Hyg Toxicol 2017;68:245–53.
2. Tenzer S, Docter D, Kuharev J et al. Rapid formation of plasma protein corona critically affects nanoparticle pathophysiology. Nat Nanotechnol 2013;8:772–81.
3. Clerc F, Reiding KR, Jansen BC et al. Human plasma protein N-glycosylation. Glycoconj J 2016;33:309–43.

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Nanomechanical tool for characterization of breast cancer cells

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Some of the commonly used medicines, such as NSAIDs, antidepressants and anticonvulsives, show potential for endocrine disruption. Interplay of these drugs with endocrine hormones can affect the status of cancers, especially those of endocrine or reproductive systems (1–4). They can have proliferative effects on cells and lead to the deterioration of patient's condition, or they can affect cancer cells negatively and therefore improve the therapy and condition. AFM is a type of scanning probe microscope and its near-field technique is based on the interaction between a sharp tip and the sample surface. This method is useful for probing the viscoelastic properties of living cells in culture and, therefore providing an indirect indicator of the structure and function of the underlying cytoskeleton and cell organelles. This method helps our understanding of cell mechanics in normal and diseased states and provides future potential in the study of disease pathophysiology and in the establishment of novel diagnostic and treatment options (5–7). The measurements were done with non-treated cells that were used as controls and the cells treated with ibuprofen in concentration of 10 μM and fluoxetine in 1 μM concentration. These compounds, as well as their concentrations were chosen for these experiments because they were shown in preliminary experiments to have estrogenic and anti-estrogenic effects. Therefore, these compounds in these concentrations affect cell proliferation of T47D-KBluc and these effects alter the mechanical properties of breast cancer cells T47D-KBluc employed in this research.

References

1. Piazza MJ, Urbanetz AA. Environmental toxins and the impact of other endocrine disrupting chemicals in women's reproductive health. J Bras Reprod Assist. 2019;23(2):154–64.
2. Lauretta R, Sansone A, Sansone M, Romanelli F, Appetecchia M. Endocrine Disrupting Chemicals: Effects on Endocrine Glands. Front Endocrinol (Lausanne). 2019;21;10.
3. Gonsioroski A, Mourikes VE, Flaws JA. Endocrine disruptors in water and their effects on the reproductive system. Int J Mol Sci. 2020;21(6).
4. McLachlan JA. Environmental signaling: from environmental estrogens to endocrine-disrupting chemicals and beyond. Andrology. 2016;4(4):684–94.
5. Kirmizis D, Logothetidis S. Atomic force microscopy probing in the measurement of cell mechanics. Int J Nanomedicine. 2010;137.
6. Mao P, Cohen O, Kowalski K, Kusiel J, Buendia J, Exman P, et al. Abstract 314: Acquired FGF and FGFR alterations confer resistance to estrogen receptor (ER) targeted therapy in metastatic ER+ breast cancer. In: Experimental and Molecular Therapeutics. American Association for Cancer Research; 2019;314–314.
7. Plodinec M, Loparic M, Monnier CA, Obermann EC, Zanetti-Dallenbach R, Oertle P, et al. The nanomechanical signature of breast cancer. Nat Nanotechnol. 2012;7(11):757–65.

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Development of Lateral Flow Assay for Detection of DOX-Resistant Breast Cancer Biomarker

D. Buse Durdabak[1], S. Dogan[2], M. Eser[3], K. Başak[4], C. O'Sullivan[5], B. Guvenc Tuna[1]

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Doxorubicin (DOX), an anthracycline, is a commonly used anticancer drug for the treatment of breast cancer. It suppresses cancer cell development by inhibiting an enzyme called topoisomerase 2b, resulting in cell death [1]. However, some cancer patients may be unresponsive to DOX. It has been reported that whilst stalling tumor growth indicates the effectiveness of an anticancer drug, its dosage may not be enough for tumor repression. On the other hand, a continuation in tumor growth is indicative of drug ineffectiveness, which could be due to the resistance of the cancer cells resistant to the medication. As previously reported, some cells, such as MCF-7 human breast cancer cells, develop resistance to DOX over time [2]. In practice, drug resistance is tested by combining any two tissue biopsies, biomarker testing, and positron emission tomography. These procedures, however, are lengthy (>3 days) and are prone to problems in accuracy and reliability. Therefore, this study aims to develop a rapid lateral flow assay for the detection of DOX-resistant breast cancer patients using microRNAs (miRNA) that are reported to be associated with the development of drug resistance. For example, the expression of miR-202-5p in the DOX/MCF-7 cell line has been reported to be approximately 2-fold higher as compared to MCF-7 cells [3]. Based on this report, miR-202-5p expression in whole blood samples of breast cancer patients (n=4), DOX-treated or DOX-untreated (Paclitaxel or Herceptin-treated), was measured by RT-qPCR, as a gold standard. The miR-202-5p was observed to be expressed to a higher level (~2-fold) in DOX-untreated breast cancer patients as compared to the DOX-treated patients. A lateral flow assay was then designed for the detection of the miR-202-5p. The assay is based on single-stranded DNA reporter probes conjugated with gold nanoparticles and a single-stranded DNA probe immobilized at the test line. Isothermal recombinase polymerase amplification using tailed primers resulted in a duplex flanked by two single-stranded tails, designed to hybridize to the reporter and capture probe.

In a conclusion, miR-202-5p could be a potential biomarker for the detection of DOX-resistant breast cancer patients. Future work will continue with nanoparticle synthesis and implementation of lateral flow assays to detect DOX-resistant cancer patients during chemotherapy.

References

[1] Thorn, C. F., Oshiro, C., Marsh, S., Hernandez-Boussard, T., McLeod, H., Klein, T. E., & Altman, R. B. (2011). Doxorubicin pathways: Pharmacodynamics and adverse effects. *Pharmacogenetics and Genomics*, *21*(7), 440–446.

[2] Smith, L., Watson, M. B., O'Kane, S. L., Drew, P. J., Lind, M. J., & Cawkwell, L. (2006). The analysis of doxorubicin resistance in human breast cancer cells using antibody microarrays. *Molecular Cancer Therapeutics*, *5*(8), 2115–2120.

[3] Liu, T., Guo, J., & Zhang, X. (2019). MiR-202-5p/PTEN mediates doxorubicin-resistance of breast cancer cells via PI3K/Akt signaling pathway. *Cancer Biology & Therapy*, *20*(7), 989–998.

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Development of an occupational risk assessment for nanobiomaterials used in Advanced Therapy Medicinal Product for cancer treatment

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The assessment of the safety of nano-biomedical products for patients is an essential prerequisite for their market authorization. However, it is also required to ensure the safety of the workers who may be unintentionally exposed to nano-biomaterials (NBMs) in these medical applications along the entire lifecycle of these products, considering not only product manufacturers but also healthcare personnel (e.g., nurses, physicians, technicians) using the products for treating patients.

In this context, three main life cycle stages (e.g., product manufacturing, use and end-of-life) and specific categories of workers for each of them were identified for the description of exposure scenarios of magnetite (Fe3O4) NBMs coated with PLGA-b-PEG-COOH used as contrast agent in magnetic resonance imaging (MRI) for the diagnosis of solid tumours. To collect information about the product manufacturing and end-of-life stages, interviews to product manufacturers and waste disposal personnel were performed, while for the use stage, a questionnaire for healthcare staff was developed and then sent to public and private clinicals and hospitals where different type of contrast agent are administered. Based on information collected, several Contributing Exposure Scenarios were identified and important considerations on workers exposure (e.g., number of people performing every single task, possible routes of exposure, duration of tasks), risk management measures used (e.g., personal protective equipment and local exhaust ventilation system) and characteristic of the room or building (e.g., dimension of the room, type of ventilation) were obtained. Moreover, a monitoring campaign was also performed at the industry where possible release during synthesis of NBMs was assessed. Monitoring measurements revealed a negligible inhalation exposure of workers potentially exposed during the production of magnetite NBMs. The exposure measurements as well as information collected were then used to perform a probabilistic risk characterisation for the formulated exposure scenarios, including uncertainty analysis.

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Characterization of Carbon nanostructures

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Graphene and carbon nanotubes are the new synthetic carbon allotropic modifications or which are some of the most extraordinary new materials of the 21st century. They feature a large specific surface, high internal mobility, high mechanical properties, high thermal conductivity, optical permeability, electrical conductivity and high potential as carriers of controlled drug release cytostatic [1-4]. The main goal of this STMS grant research is: 1. Production of graphene and carbon nanotubes by electrolysis in molten salts applying non-stationary current regimes at the Faculty of Technology and Metallurgy in Skopje, and 2. Characterization of synthesized carbon nanostructures using scanning electron microscopy (SEM), transmission electron microscopy (TEM), Raman spectroscopy at the Faculty of Technology and Metallurgy in Belgrade, Serbia [5-8]. These techniques are of great importance to characterize carbon nanostructures, or more precisely to determine certain properties important for their further use for the purposes of the COST Action CA17140: Cancer Nanomedicine – from the Bench to the Bedside. Microscopic images have been obtained showing that the synthesized carbon nanostructures are of high quality. The graphene is composed of several layers with a thickness of 0.2 to 2 nanometers, and carbon nanotubes are MWCNT's with a diameter of 5 to 30 nanometers, whereas the lengths of both materials are in micrometer dimensions.

References

1. A.T. Dimitrov, G.Z. Chen, I.A. Kinloch and D.J. Fray, A Feasibility study of Scaling-up the Electrolytic Production of Carbon Nanotubes in Molten Salt, Electrochimica Acta 48 (2002) 91-102.
2. K. S. Novoselov, A. K. Geim, S. V. Morozov, D. Jiang, Y. Zhang,Y. S. V. Dubonos, I. V. Grigorieva and A. A. Firsov, Science, 2004, 306, 666; K. S. Novoselov, A. K. Geim, S. V. Morozov, D. Jiang, M. I. Katsnelson, I. V. Grigorieva, S. V. Dubonos and A. A. Firsov, Nature, 2005, 438, 197; C. N. R. Rao, K. Biswas,K. S. Subrahmanyam and A. Govindaraj, J. Mater. Chem., 2009,19, 2457.
3. K. S. Novoselov, D. Jiang, F. Schedin, T. J. Booth, V. V. Khotkevich, S. V. Morozov and A. K. Geim, Proc. Natl. Acad. Sci. U. S. A., 2005, 102, 10451.
4. Aleksandar T. Dimitrov, Study of Carbon Nanotubes Production by Electrolysis in Molten Li2CO3, Macedonian Journal of Chemistry and Chemical Engineering, Vol. 28, No. 1, pp. 111–118 (2009).
5. C.Schwandt, A.T.Dimitrov, D.J.fray, The preparation of nano-structured carbon materials by electrolysis of molten lithium chloride at graphite electrodes, Journal of Electroanalytical Chemistry 647 (2010)150-158.
6. Carsten Schwandt, Aleksandar T. Dimitrov, Derek J. Fray, High-yield synthesis of multi-walled carbon nanotubes from graphite by molten salt electrolysis,CARBON, 50 (2012) 1311–1315
7. B. Andonovic, A. Grozdanov, P. Paunović, A. T. Dimitrov, X-ray diffraction analysis on layers in graphene samples obtained by electrolysis inmolten salts: a new perspective, Micro & Nano Letters, 10 (12), 683-685 (2015)
8. B. Andonović, A. Grozdanov, A. Petrovski, P. Paunović, A. Dimitrov, Determining graphene layers number andN-layer region coverage by XRD data distribution model, Material and Environment Protection, 4(1), 19-25 (2015)

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Carbosilane Dendritic Amphiphiles for Cancer Nanomedicine Drug Delivery

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Supramolecular nanostructures such as micelles, liposomes, polymersomes or dendrimersomes are widely studied and used as drug delivery systems. The behavior of their amphiphilic building blocks strongly depends on spatial distribution and shape of polar and nonpolar component. In this project, we studied a series of amphiphilic carbosilane dendrons bearing various polar groups. Their polar part is formed by different number of dendritic wedges and their nonpolar part is formed by a different number of alkyl chains of different lengths (See Figure 1). After the estimation of their critical micelle concentrations, sizes of nanoparticles formed in different aqueous solutions were measured by dynamic light scattering. Studied substrates form micelles at almost all conditions. These micelles might be able to encapsulate and deliver lipophilic drugs inside their lipophilic environment. Positively charged dendrons also form complexes with anti-miRNA. These complexes show sizes and polydispersity favorable for delivery.

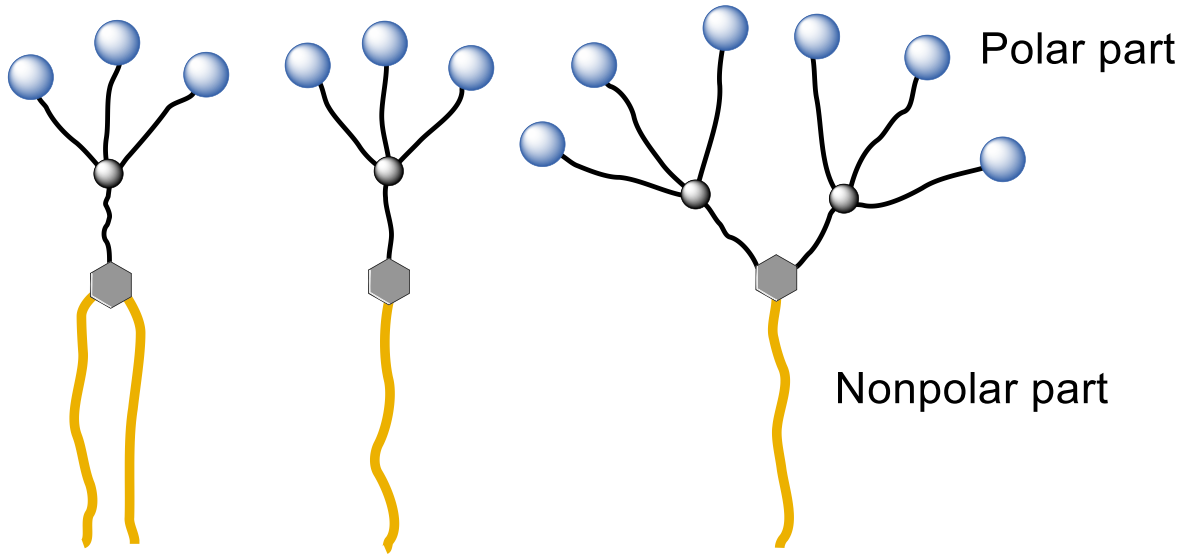

Figure 1. Schematical representation of studied dendrons.

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Synthesis and evaluation of phenanthroline-based derivatives as human pre-MIR150 G-quadruplex binders for lung cancer therapy

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The human *MIR150* is significantly upregulated in Non-Small Cell Lung Cancer (NSCLC) and has been reported to have an important role in NSCLC development [1-3]. Thus, the control of mature *MIR150* levels can provide a strategy to fight NSCLC. The formation of G-quadruplex (G4) structures in the stem-loop region of pre-miRNAs can interfere with Dicer activity and decrease the mature miRNA production inside the cell [4]. Recently, we reported that the human precursor of *MIR150* (*pre-MIR150*) folds into a G4 structure [5], which could regulate microRNA levels, thus unveiling a new potential anticancer strategy. In this context, we have synthesized and characterized different phenanthroline-based derivatives with the aim of binding and stabilizing the G4 motif present in *pre-MIR150*. The interaction of these ligands with the G4 motif has been evaluated by biophysical methods; these compounds showed moderate activity in terms of thermal stabilization. We explored different derivatives and assessed their ability to interact with the G4 motif present in *pre-MIR150*, thus providing invaluable information about structure-activity relationships (SAR).

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References

1. Jiang K, Shen M, Chen Y, Xu W. miR-150 promotes the proliferation and migration of non-small cell lung cancer cells by regulating the SIRT2/JMJD2A signaling pathway. Oncol. Rep. 2018, 40, 943-951.
2. Li H, Ouyang R, Wang, Z, Zhou W, Chen H, Jiang Y, Zhang Y, Li H, Liao M., Wang W, Ye M, Ding Z, Feng X, Liu J, Zhang B. MiR-150 promotes cellular metastasis in non-small cell lung cancer by targeting FOXO4. Sci. Rep. 2016, 6, 39001
3. Donnem T, Fenton C. J, Lonvik K, Berg T, Eklo K, Andersen S, Stenvold H, Al-Shibli K, Al-Saad S, Bremnes R. M, Busund L.-T, MicroRNA Signatures in Tumor Tissue Related toAngiogenesis in Non-Small Cell Lung Cancer. MicroRNA Signatures in Tumor Tissue Related to Angiogenesis in Non-Small Cell Lung Cancer. PLoS ONE. 2012, 7, 29671.
4. Arachchilage G. M, Dassanayake A.C, Basu S. A Potassium Ion-Dependent RNA Structural Switch Regulates Human Pre-miRNA 92b Maturation. Chem. Biol. 2015, 22, 262–272.
5. Figueiredo J, Miranda A, Lopes-Nunes J, Carvalho J, Alexandre D, Valente S. Mergny J.-L, Cruz C. Targeting nucleolin by RNA G-quadruplex-forming motif. Biochem. Pharmacol. 2021,189, 114418.

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Stem cells as in vitro model for testing nanodrug delivery properties of selenium nanoparticles

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Nanotechnology enabled design of selenium nano form, with enhanced biological activity and reduced toxicity. Specifically, selenium nanoparticles (SeNPs) have attracted significant interest due to the simple and efficient preparation, along with favorable properties [1]. The growing number of published data originating from *in vitro* studies of selenium compounds highlight its promising anticancer effects [2,3]. The development of SeNP-based chemotherapeutics with the potential to cross the blood-brain-barrier (BBB) is of particular research interest. However, brain-acting therapeutics require extensive toxicity testing prior to their clinical application. As induced pluripotent stem cells (iPSC) are a powerful *in vitro* model, this STSM was focused on the use of iPSC line generated from a Parkinson's disease patient carrying a mutation in LRRK2 gene. The cells were characterized by abnormal expression of alpha synuclein, protein identified as one of the factors in Parkinson disease and tumorigenesis [4,5]. When the SP12 iPSC cells reached 80 % confluency, embryoid bodies (EBs) were generated. Then, dopaminergic neurons were successfully generated, as confirmed by staining the cells with antibodies specific for tyrosine hydroxylase (TH) and neuron-specific class III beta-tubulin (TUJ1). The neurons were then treated with SeNPs and evaluated by immunofluorescence method. The neurons were stained with fluorescently labeled antibodies for TH and Alpha-synuclein, and DAPI was used to counterstain for nuclei. iPSC-derived neuronal cells were sensitive to treatment with SeNPs, which caused apoptosis in majority of the cells.

References

1. Hosnedlova B, Kepinska M, Skalickova S, Fernandez C, Ruttkay-Nedecky B, Peng Q, Baron M, Melcova M, Opatrilova R, Zidkova J, Bjørklund G, Sochor J, and Kizek R. Nano-selenium and its nanomedicine applications: a critical review. Int. J. Nanomedicine. 2018; 13: 2107–2128.
2. Huang Y, He L, Liu W, Fan C, Zheng W, YWong Y. S, Chen T. Selective cellular uptake and induction of apoptosis of cancer-targeted selenium nanoparticles. Biomaterials. 2013; 34: 7106–7116.
3. Yu B, Zhang Y, Zheng W, Fan C, Chen T. Positive surface charge enhances selective cellular uptake and anticancer efficacy of selenium nanoparticles. Inorg. Chem. 2012; 51: 8956–8963.
4. Israeli E, Yakunin E, Zarbiv Y, Hacohen-Solovich A, Kisos H, Loeb V, Lichtenstein M, Ben-Gedalya T, Sabag O, Pikarsky E, Lorberboum-Galski H, Sharon R, α-Synuclein expression selectively affects tumorigenesis in mice modeling Parkinson's disease. PLoS One. 2011; 6: e19622.
5. Sánchez-Danés A, Richaud-Patin Y., Carballo-Carbajal I, Jiménez-Delgado S, Caig C, Mora S, Di Guglielmo C, Ezquerra M, Patel B, Giralt A, Canals J.M, Memo M, Alberch J, López-Barneo J, Vila M, Cuervo A.M, Tolosa E, Consiglio A, Raya A. Disease-specific phenotypes in dopamine neurons from human iPS-based models of genetic and sporadic Parkinson's disease EMBO Mol. Med., 2012, 4, 380

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Manual positioning device for FUS induced BBB disruption in mice

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In the last decade, many studies involving the use of mouse models have shown that focused ultrasound (FUS) administered with an ultrasound contrast agent can disrupt the Blood Brain Barrier (BBB) so that molecules of pharmacologically relevant size can enter the brain parenchyma [1-3]. Herein, a 2 degree of freedom manual robotic device intended for such experiments is presented. The device was entirely manufactured on a 3D printing machine (FDM 270, Stratasys, Minnesota, USA) using the fused disposition modeling technique. Figure 1 shows computer-aided design drawings of the device with the main components indicated. The positioning mechanism is dedicated to moving a single element spherically focused ultrasonic transducer along two orthogonal axes X and Y in the horizontal plane, with an available motion range of 60 and 130 mm, respectively. The user can easily adjust the transducer's position manually using adjustment knobs that are located at the front of the device. The rotational motion of the X-axis knob is directly converted into linear motion by a jack screw mechanism. For linear motion in the Y-axis, bevel gears were incorporated for transferring motion at a right angle to the respective jack screw. The transducer and all the moving parts are accommodated in a compact enclosure that includes an acoustic opening at the top. A special holder featuring four moving plates with locking levers was designed and fitted in the acoustic opening to safely immobilize mice of any size and type. During operation the enclosure is filled with degassed water that serves as the coupling medium for proper beam propagation from the transducer to the mouse. Overall, the proposed device constitute a cost-effective and ergonomic solution for FUS mediated BBB disruption in mice.

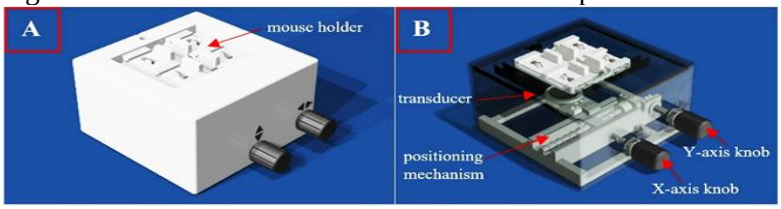

Figure 1. CAD drawings of the positioning device with A) opaque and B) transparent covers.

References

1. Choi James J., Wang Shougang, Tung Yao-Sheng, Morrison 3rd Barclay, Konofagou Elisa E. Molecules of various pharmacologically-relevant sizes can cross the ultrasound-induced blood-brain barrier opening in vivo. Ultrasound Med Biol. 2010; 36:58-67. 2. Lapin Norman A., Gill Kirt, Shah Bhavya R., Chopra Rajiv. Consistent opening of the blood brain barrier using focused ultrasound with constant intravenous infusion of microbubble agent. Sci Rep. 2020; 10. 3. Choi James J, Selert Kirsten, Gao Zimeng, Samiotaki Gesthimani, Baseri Babak, Konofagou Elisa E. Noninvasive and localized blood-brain barrier disruption using focused ultrasound can be achieved at short pulse lengths and low pulse repetition frequencies. J Cereb Blood Flow Metab. 2011; 31:725-737.

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Complexation properties of glycodendrimers towards nucleotides

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Our project aimed at application of sugar-modified poly(propyleneimine) (PPI) dendrimers as drug delivery devices for adenosine analogue anticancer drugs (clofarabine and fludarabine), in order to shield them from biological and chemical degradation, help to overcome various resistance mechanisms and enable the delivery of active forms of these therapeutics. The premise of our work is the use of non-covalent drug/dendrimer complexes. The main goal of the STSM project involved the comprehensive characterization of stability of fludarabine-dendrimer and clofarabine-dendrimer complexes in different pH conditions. This was achieved by the application of asymmetrical flow field-flow fractionation (AF4), routinely used in research conducted by the team from Leibniz Institute of Polymer Research Dresden. This method provided a crucial supplement to our previous studies, giving a complete picture of the formation and stability of the dendrimer-drug complexes. In line with our previous results, AF4 experiments proved that nucleotides form stable complexes with cationic PPI dendrimers due to the electrostatic interactions between negatively charged phosphate tails of nucleotides and positive amino groups of dendrimer macromolecules. Nucleotide-dendrimer complexation was pH-dependent, being most efficient in acidic environment. Under such conditions, the degree of protonation of primary and tertiary amino groups of the dendrimer increases, and thus the number of potential nucleotide binding sites. Upon the increase of pH, PPI dendrimers bound less nucleotide molecules, most likely due to deprotonation of macromolecule amino groups. The stability of the complexes depended significantly on the drug-dendrimer complexation ratios. Considering that the nucleotide-dendrimer molar ratios of 20:1 for PPI G4 and 10:1 for PPI-Mal OS G4 (due to the partial maltose coating) allow for the complete saturation of polymers with triphosphate molecules, it is not unexpected that for these and lower ratios (10:1 and 5:1 for PPI G4 and PPI-Mal OS G4, respectively) approximately 100% complexation was observed. The percentage of complexed drug molecules decreased with increasing drug-dendrimer ratio. Most importantly, clofarabine triphosphate was interacting more strongly with PPI dendrimers than fludarabine triphosphate (more CAFdATP molecules were bound with the dendrimer under the same conditions). Such a trend has been observed consistently for all buffers and the majority of drug:dendrimer ratios. This outcome supports the hypothesis that the efficient release of CAFdATP from complex with the dendrimer may be hampered, thus decreasing the delivery potential of PPI macromolecules in case of this drug.

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Advanced High-Affinity Glycocluster Ligands of Galectins

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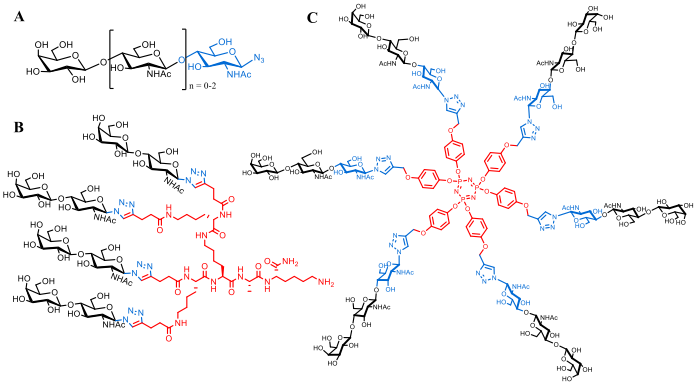
Galectins are proteins belonging to the family of human lectins. By binding galactose units of glycans on cell surfaces, they participate in biological and pathological processes like cell signaling, cell adhesion, apoptosis, fibrosis, cancerogenesis, and metabolic disorders. The binding of monovalent glycans to galectins is usually relatively weak, and, therefore, the presentation of carbohydrate ligands on multivalent scaffolds is efficient for increasing the glycoconjugate affinity to various galectins [1]. A library of glycoclusters and glycodendrimers (see Figure 1) with various structural presentations of the functionalized *N*-acetyllactosamine (LacNAc) ligand was prepared to show the effect of presentation on the affinity and selectivity for two most abundant galectins, galectin-1 (Gal-1) and galectin-3 (Gal-3). Moreover, two chito-LacNAc linkers were prepared using mutant β-*N*-acetylhexosaminidase from *Talaromyces flavus* and β-galactosidase BgaD from *B. circulans* [2], and their impact on the glycoconjugate affinity was determined. A new design of biolayer interferometry (BLI) method with specific AVI-tagged constructs was used for the affinity determination and compared to the gold standard method of isothermal titration calorimetry (ITC). This study reveals new pathways to low nanomolar glycoconjugate inhibitors of galectins, prospective for biomedical research.

Figure 1. **A**. Chito-LacNAc ligand, **B.** and **C.** examples of glycopeptides with chito-LacNAc



References

1.Heine V, **Hovorková M**, *et al. Eur J Med Chem*., 2021, 220: 113500. 2. **Hovorková M**, *et al*. *ChemCatChem*, 2021, 13: 4532-4542.

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A microfluidic model of the kidney – a platform for the determination of renal toxicity

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Simulating realistic *in vivo* conditions for *in vitro* research in the laboratory to answer complicated biological questions is the basic step in each biomedical research. The traditional approach is cultivating cells in culture media in flasks or Petri dishes under static conditions. However, conditions inside the body are not static; actually, they are very dynamic. Microfluidic technology is a platform that offers the more physiologically-relevant conditions for *in vitro* research.

The main organ of the body detoxification is the kidneys. As they filtrate the whole blood, they are often exposed to toxic substances. These vital organs maintain the body's homeostasis and eliminate various xenobiotics, including drugs and nanomaterials. Therefore, an adequate kidney model to study the potential nephrotoxic effect of new drugs needs to be developed. The Fraunhofer IBMT has deep experience with developing advanced in vitro models for toxicology screening and lot of cell barrier models are there routinely used. The STSM allows me to acquire new skills and knowledge in the state-of-the-art cell cultivation.

In our study, the new experimental microfluidic system was employed as a screening tool for renal cells. TH-1 cells, human renal epithelial cell line, were cultivated in the microfluidic module under constant medium flow rate and exposed to cisplatin (known nephrotoxic compound) and fluorescent-labeled silica nanoparticles. The cells were continuously optical observed and vital stained after treatment. The standard static cultivation method has been used as a comparison. The results show that the cell morphology and proliferation activity haven't been affected by cultivation in a microfluidic module, and we were able to detect changes after treatment with nephrotoxic substance; therefore, the microfluidic system could be a new promising platform for toxicity screening.

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Application of auto–fluorescent nanoparticles as a new bioimaging agents *in vivo*

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As we know from our previous studies, some dendrimers possess auto–fluorescence [1,2]. This time, a different type of auto-fluorescent nanoparticles was used for the research, namely carbon quantum dots (CQD). Thanks to auto–fluorescence, CQD can be analyzed in cells by confocal microscopy and flow cytometry without additional labeling. It has been formed a hypothesis that a zebrafish would serve as a suitable model to perform the biodistribution study of auto–fluorescent nanoparticles and that it will be possible to document the results by confocal microscopy. In addition, an acute fish toxicity test was made using the fish embryo test (FET). According to the procedure, newly fertilized zebrafish embryos were exposed to the tested CQD's for a total of 96 h. Every 24 h, up to four apical observations were recorded as indicators of lethality: (1) coagulation of fertilized eggs, (2) lack of somite formation, (3) lack of detachment of the tail bud from the yolk sac, and (4) lack of heartbeat. The obtained results indicate that the carbon quantum dots are surprisingly more toxic to embryos even in chorion than to HeLa cells and cause coagulation of fertilized eggs. What is interesting, less toxic sucrose-tartrate quantum dots also unexpectedly helped to hatch the fish from the chorion (egg shell) 24 hours earlier. Microscopy analysis of trafficking/localization of auto–fluorescent nanoparticles *in vivo* show CQD's concentration in the nervous system after 72h of incubation. Our research confirmed the hypothesis that zebrafish is a suitable model for the study of the biodistribution of auto-fluorescent nanoparticles such as carbon quantum dots.

References

1. Janaszewska A, Studzian M, Petersen JF, Ficker M, Paolucci V, Christensen JB, Tomalia DA, Klajnert-Maculewicz B. Modified PAMAM dendrimer with 4-carbomethoxypyrrolidone surface groups-its uptake, efflux, and location in a cell. Colloids and Surfaces B: Biointerfaces. 2017;159:211–216.
2. Tomalia DA, Klajnert-Maculewicz B, Johnson KAM, Brinkman HF, Janaszewska A, Hedstrand DM. Non-traditional intrinsic luminescence: inexplicable blue fluorescence observed for dendrimers, macromolecules and small molecular structures lacking traditional/conventional luminophores. Progress in Polymer Science.2019;90:35–117

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Dendrimers for microRNA delivery into human glioblastoma stem-like cells

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Background: Glioblastoma is supposed to be one of the most aggressive and hard-to-treat type of tumor. Importantly, recent research advocates that glioblastoma progression is mostly driven by tumor cells with stem cell properties – glioblastoma stem-like cells (GSCs) [1]. Targeting this subpopulation could be an effective way to treat glioblastoma, getting over the drug resistance. We suggest novel approach, based on using cationic dendritic molecules, which proved their antitumor properties in previous experiments on other models [2–4], as carriers for microRNAs with antitumor activity – pro-apoptotic microRNA-34a [5] and a synthetic inhibitor of one of the most well-known oncomiR microRNA-21a [6]. **Experimental**: In our study we used amphiphilic triazine-carbosilane dendrones of the 2nd (DG2) and the 3rd (DG3) generations, fully symmetrical carbosilane (BDEF33) and phosphorus (AE2G3) dendrimers of the 3rd generation. Several GSCs lines were chosen: BTSC233, JHH520, GBM1 and NCH644, as well as standard U87 glioblastoma cell culture in suspension state. iPS cells were used as non-tumor control. To prove the dendriplexes can be sufficiently internalized, we performed FACS analysis after 3 h of incubating cells with FITC-labelled microRNA. On the next step cells were cultivated with free dendrimers or complexes (25, 50, 100, 150 nM RNA, 10-fold excess of cations) for 72 h. We investigated cell viability (MTT assay, Annexin V/PI apoptosis assay), IL-10 secretion and expression of several markers, characterizing interaction of tumor with immune microenvironment - PD-L1, TIM-3, CD47. Statistical analysis was done by using Mann-Whitney and Wilcoxon tests, differences between groups were considered significant if $p<0.05$. **Results:** Free dendrimers have their own dose-dependent toxic effects, which is higher for tumor cells, than iPS. Interestingly, toxic effect of dendrimers was higher than after temozolomide treatment in case of GSCs cultures. Treatment by free dendritic molecules led to increasing of expression of PD-L1 and TIM-3 on NCH644 cells, but decreasing the PD-L1 expression on GBM1 cells. IL-10 secretion was intact. Dendriplexes can be efficiently internalized into tumor cells, they had pro-apoptotic effect on glioblastoma cells. PD-L1 expression did not change (U87) or decreased (GBM1) after treatment by dendriplexes. **Conclusion:** not only have their own toxic effect per se, but also they can be used as effective nanocarriers for therapeutic microRNAs We suppose, that dendrimer-based approach can be useful and perspective tool for delivery of therapeuthic microRNAs into tumor cells. At the same moment, characterization of their effects on interactions between tumor and immune microenvironment demands the further studies.

References

[1] U.D. Kahlert, S.M. Mooney, M. Natsumeda, H.-J. Steiger, J. Maciaczyk, Targeting cancer stem-like cells in glioblastoma and colorectal cancer through metabolic pathways, Int. J. Cancer. 140 (2017) 10–22.. [2] N. Knauer, E. Pashkina, E. Apartsin, Topological aspects of the design of nanocarriers for therapeutic peptides and proteins, Pharmaceutics. 11 (2019).. [3] M.A. Mintzer, M.W. Grinstaff, Biomedical applications of dendrimers: A tutorial, Chem. Soc. Rev. 40 (2011) 173–190.. [4] B. Klajnert-Maculewicz, L. Peng, V. Cena, Dendrimers in Biomedical Applications, Royal Society of Chemistry, Cambridge, 2013. [5] M. Agostini, R.A. Knight, miR-34: from bench to bedside, Oncotarget. 5 (2014) 872–881. [6] C.H. Lawrie, MicroRNAs and lymphomagenesis: a functional review, Br. J. Haematol. 160 (2013) 571–581.

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Structure of mixed-monolayer gold nanoparticles: Molecular Dynamics study

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One of the advantages of using gold nanoparticles (AuNPs) as drug carriers is the extensive range of their functionalization. Their surface properties allow for the introduction of large number of different ligand types. In theory, by choosing ligands with specific physico-chemical properties, we can modify AuNPs structure to "program" the desired physiological behavior. However, to actually do it, we need to understand how these ligands interact with each other and their environment, and how can we exploit these interactions to achieve the desired effect.
Introducing even slight changes to the existing structures can create a large number of interactions whose impact on the structure is difficult to predict. Investigating this experimentally is expensive, and extremelly difficult since most experimental methods lack the necessary resolution on such small scales. However, simulations done with the desired precision can provide useful insights.
In this study, we ran atomistic molecular dynamics simulations of mixed-monolayer gold nanoparticles. The aim was to investigate how the changes in the intitial composition affect the overrall structure of functionalized AuNPs. We have simulated multiple systems, changing only the carried drugs and their concentration in the monolayer, while keeping all the other parameters fixed to assure that the observed effects are solely the result of these changes. Results showed that even such narrowly defined changes highly influence the overall structure of equilibrated functionalized AuNPs.

In summary, our results indicate that careful consideration of physico-chemical properties of both carried drugs and the entire nano-system should be taken into account when designing functionalized AuNPs for a specific purpose.

References

1. Kovacevic M, Balaz I, Marson D, Laurini E, Jovic B. Mixed-monolayer functionalized gold nanoparticles for cancer treatment: Atomistic molecular dynamics simulations study. BioSystems. 2021; 202, 104354

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Preclinical characterization of biocompatible multifunctional nanodevices for cancer nanomedicine

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Cancer nanomedicine has become a promising therapeutic approach to overcome the limitations of conventional chemotherapeutics by improving drug internalization and selective intracellular accumulation in cancer cells, easing the toxicity to normal tissues [1]. 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 [2]. Recently, we have designed several polymeric nanoparticles for selective drug delivery, theranostic and sensing [3,4]. Herein we reported the results of the preclinical evaluation of this kind of multifunctional nanodrugs, focusing on running toxicity assays.

Three nanodevices were studied: (i) a non-engineered nanoparticle which is amino-functionalized (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).

References:

[1] Chen G, *et al.* Nanochemistry and Nanomedicine for Nanoparticle-based Diagnostics and Therapy. Chem. Rev. 2016, 116, 2826–2885

[2] Rosenblum D, *et al*. Progress and challenges towards targeted delivery of cancer therapeutics. Nat Commun. 2018 Apr 12;9(1):1410. doi: 10.1038/s41467-018-03705-y

[3] Cano-Cortes MV, *et al*. Characterization and Therapeutic Effect of a pH Stimuli Responsive Polymeric Nanoformulation for Controlled Drug Release. Polymers (Basel). 2020 Jun 1;12(6):1265. doi: 10.3390/polym12061265

[4] Laz-Ruiz JA, *et al.* An effective polymeric nanocarrier that allows for active targeting and selective drug delivery in cell coculture systems. Nanoscale. 2021 Feb 18;13(6):3500-3511. doi: 10.1039/d0nr07145e

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ICG-tagged aptamer as drug delivery system for malignant melanoma

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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 exist different therapeutic options for melanoma treatment, such as surgery, chemotherapy, radiotherapy, photodynamic therapy and immunotherapy [2,3]. 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 [4]. 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 [5]. Thus, we synthesized and evaluated a novel drug delivery system composed of AS1411 and indocyanine green (ICG) to track its accumulation in tumoral cells in a melanoma mouse model. Using a simple supramolecular strategy, we conjugated the complex AS1411-ICG with C8 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. The circular dichroism (CD) data suggest that C8 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 C8 increases the cytotoxicity compared with AS1411-ICG alone. From the *in vivo* studies, we conclude that both AS1411-ICG and AS1411-ICG-C8 presented the potential to accumulate preferentially in tumor tissues. Moreover, these compounds seem to be efficiently removed from the mice's bodies through kidney clearance. In summary, these results suggest that these complexes derived from AS1411 aptamer could act as a delivery system of ligands with antitumoral activity for *in vivo* melanoma therapy.

References

1. Sandru A, Voinea S, Panaitescu E, Blidaru A. Survival rates of patients with metastatic malignant melanoma. J. Med. Life. 2014; 7: 572-576. 2. Naidoo C, Kruger C A, Abrahamse H. Photodynamic therapy for metastatic melanoma treatment: a review. Technol. Cancer Res. Treat. 2018; 17: 1-15. 3. Lugowska I, Teterycz P, Rutkowski P. Immunotherapy of melanoma. Contemp. Oncol. 2018; 22: 61-67 4. Bhatia S, Tykodi S S, Thompson J A. Treatment of metastatic melanoma: an overview. Oncology. 2009; 23: 488-496. 5. Bates P J, Reyes-Reyes E M, Malik M T, Murphy E M, O'Toole M G, Trent J O. G-quadruplex oligonucleotide AS1411 as a cancer-targeting agent: uses and mechanisms. Biochim. Biophys. Acta – Gen. Subj. 2017; 1861: 1414-1428.

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Carbosilane glycodendrimers for anticancer drug delivery

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The complexity of drug delivery mechanisms still encourages innovations in the molecular transport systems [1,2]. In the scope of STMS, we developed a robust synthetic method for the preparation of carbosilane dendritic structures enabling multivalent presentation of sugar ligands and other biochemically relevant compounds. Regardless of series or generation, the dendrimers peripherally decorated with glucose and galactose derivatives exhibited outstanding biocompatibility with all tested cell lines indicating their potential as nanocarriers in drug delivery. These findings led to the preparation of stable glycodendrimer complexes with doxorubicin (glyco-DDM/DOX), a highly toxic substance with potent anticancer activity. *In vitro* cytotoxicity assay of the complexes revealed generation and concentration-dependent effects across the tested cell lines, but most importantly, their general selectivity toward cancer cell lines. Moreover, glyco-DDM/DOX complexes were much less internalized in non-cancerous cells compared to cancer cell lines. Higher IC_{50} values of the majority of the complexes compared to pure doxorubicin showed that the peripheral saccharide units substantially decreased the cytotoxicity of the glyco-DDM/DOX complexes. Thus, for the benefit of cancer therapy, a higher dose of doxorubicin can be introduced using the glyco-DDM nanocarriers, whereas slower drug release stabilizes the concentration of the therapeutic agent in cancer cells. In addition, the doxorubicin release was up to 2−3 times faster in acidic environments than under physiological conditions, indicating preferential drug release in the vicinity of tumor tissues [3].

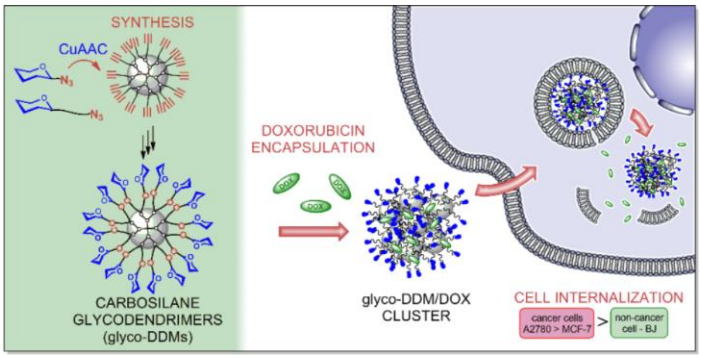

Figure 1. Synthesis of carbosilane glycodendrimers, doxorubicin encapsulation and drug release.

1. Liegertova, M., et al. Evaluation of toxicological and teratogenic effects of carbosilane glucose glycodendrimers in zebrafish embryos and model rodent cell lines. *Nanotoxicology*, 2018, **12**(8): p. 797-818. 2. Muller, C., et al. Organizing multivalency in carbohydrate recognition. *Chemical Society Reviews*, 2016, **45**(11): p. 3275-3302. 3. Müllerová, M. et al. Carbosilane glycodendrimers for anticancer drug delivery: Synthetic route, characterization, and biological effect of glycodendrimer−doxorubicin complexes. *Biomacromolecules*, 2021, **23**(1): p. 276–290.

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Influence of PAMAM-taxanes-monoclonal antibody conjugates on the breast cancer cells

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Breast cancer is one of the most common malignancies in women. It has been confirmed that approximately 30% of female patients have overexpression of human epidermal growth factor 2 (HER2) on the surface of tumor cells. Trastuzumab is a recombinant, humanized monoclonal antibody directed against this receptor. Its use in traditional chemotherapy causes an increase of therapy efficiency. However, the systemic toxicity of the anticancer drugs is still a serious problem[1].

Dendrimers have been proven to improve the drugs solubility and also ensure their gradual release in the tumor environment. In addition, the presence of conjugated trastuzumab ensures effective drug transport to HER2-overexpressed cells. Therefore, PAMAM-paclitaxel and PAMAM-docetaxel conjugates were synthesized, and thanks to the monoclonal antibody attached to them, whole system would have the ability to act selectively in the treatment of the HER2 overexpressing breast cancer.

To confirm the effectiveness and selective cytotoxicity of the conjugates of PAMAM-drug-trastuzumab, their cytotoxicity was assessed and the IC50 parameter was determined for two breast cancer cell lines (SKBR-3 HER2-positive and MCF-7 HER2-negative). The obtained results showed selectivity of conjugates towards cells overexpressing HER2 receptor. The confocal microscopy confirmed intracellular location of the analyzed compounds. In order to identify a system with the greatest therapeutic potential, mechanisms of action of the PAMAM dendrimer conjugates were compared. For this purpose, the level of reactive oxygen species, changes in mitochondrial potential, activation of caspases, induction of apoptosis and cell cycle phase distribution were determined. Obtained results have shown the complexity of the cytotoxic mechanism of PAMAM-drug-trastuzumab conjugate activity[2].

References

1. Marcinkowska, M., Sobierajska, E., Stanczyk, M., Janaszewska, A., Chworos, A., Klajnert-Maculewicz, B. Conjugate of PAMAM dendrimer, doxorubicin and monoclonal antibody-trastuzumab: The new approach of a well-known strategy (2018) Polymers, 10 (2), art. no. 187, . Cited 14 times. DOI: 10.3390/polym10020187, IF 8.183
2. Marcinkowska, M., Stanczyk, M., Janaszewska, A., Sobierajska, E., Chworos, A., Klajnert-Maculewicz, B. Multicomponent Conjugates of Anticancer Drugs and Monoclonal Antibody with PAMAM Dendrimers to Increase Efficacy of HER-2 Positive Breast Cancer Therapy (2019) Pharmaceutical Research, 36 (11), art. no. 154, Cited 9 times. DOI: 10.1007/s11095-019-2683-7, IF 3.90

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Development of aptadendrimers for prostate cancer therapy

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Prostate cancer (PCa) is the second most common cancer type among men worldwide and the most prevalent in the men community in Portugal. Due to this, is of utmost importance to develop new technologies which can be a benefit for a society heavily affected by cancer disease.

The overexpression of the protein nucleolin (NCL) has been reported on the cell surface of PCa cells, which makes it a potential cancer-selective target. NCL specifically binds G-rich sequences, that can fold into a G-quadruplex conformation like the AS1411 aptamer.

Clinical trials have already demonstrated the promising therapeutic activity of AS1411. It demonstrated great safety, but low potency and suboptimal pharmacokinetics, being rapidly cleared out from the body [1]. However, there are some reports of AS1411 derivatives (AT11, AT11-L0, AT11-B0) with promising applications in drug delivery carriers, due to its safety profile and ability to induce durable responses in some cancer cells [2] and higher drug accumulation into cancer cells than in the normal ones, due to the NCL targeting [3].

In this work, we propose to synthetize and characterize dendrimers functionalized with a AS1411 derivative (aptadendrimer), namely AT11 for a selective delivery in PCa cells having NCL overexpressed on their surface. The obtained aptadendrimers, displaying a multivalent presentation of AT11, will be loaded with a ligand and the biological effect of the proposed aptadendrimer nanocarrier will be evaluated *in vitro*.

References

[1] P. J. Bates, E. M. Reyes-Reyes, M. T. Malik, E. M. Murphy, M. G. O'Toole, and J. O. Trent, 'G-quadruplex oligonucleotide AS1411 as a cancer-targeting agent: Uses and mechanisms', Biochim. Biophys. Acta - Gen. Subj., vol. 1861, no. 5, pp. 1414–1428, May 2017, doi: 10.1016/j.bbagen.2016.12.015.

[2] W. Alshaer, H. Hillaireau, and E. Fattal, 'Aptamer-guided nanomedicines for anticancer drug delivery, Advanced Drug Delivery Reviews, vol. 134. pp. 122–137, 2018.

[3] Y. Shieh, S. Yang, M. Wei, and M. Shieh, 'Aptamer-Based Tumor-Targeted Drug', ACS Nano, vol. 4, no. 3, pp. 1433–1442, 2010.

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Fucoidan/dendrimer nanoparticles for glioblastoma treatment: *in vitro* siRNA delivery studies

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Glioblastoma multiforme (GBM) is the most common primary brain tumor among adults, which current treatment remains a significant challenge. Currently, first-line treatments include resection, radiotherapy and systemic chemotherapy with temozolomide [1]. Despite enormous advances in therapeutic modalities for this kind of malignant tumor, it remains with a poor prognosis and is mostly incurable, with patients having a life expectancy of around 14-15 months after diagnosis [2]. The low success in treating these tumors can be attributed to different biological barriers, such as the blood-brain barrier (BBB), which represents an obstacle to the crossing and effective delivery of therapeutic agents in brain tissues. Nanotechnology can constitute a solution for the efficient delivery of therapeutic agents into glioblastomas tumors.
In this STSM, we conducted a series of experiments to evaluate the possibility of using Fucoidan/dendrimer nanoparticles to deliver siRNA in glioblastoma tumors. For this purpose, the nanoparticles capacity to condensate and protect the siRNA from degradation was first studied. Then, a series of *in vitro* experiments were conducted in glioblastoma cell lines to study the nanoparticles cytotoxicity and their capability as siRNA delivery agents in the knock-down of target proteins. We could conclude that the nanoparticles could condense and protect the siRNA from RNases action. Although not showing considerable cytotoxicity, it was not possible to obtain robust results from the transfection studies. Further studies are needed to assess the capability of these nanoparticles as siRNA delivery agents in glioblastoma tumors.

Acknowledgments
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References

[1] Thakkar JP, Dolecek TA, Horbinski C, Ostrom QT, Lightner DD, Barnholtz-Sloan JS, Villano JL. Epidemiologic and molecular prognostic review of glioblastoma. Cancer Epidemiol Biomarkers Prev 2014;23:1985–96.

[2] Ostrom QT, Bauchet L, Davis FG, Deltour I, Fisher JL, Langer CE, Pekmezci M, Schwartzbaum JA, Turner MC, Walsh KM, Wrensch MR, Barnholtz-Sloan JS. The epidemiology of glioma in adults: A state of the science review. Neuro Oncol 2014;16:896–913.

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Customizing the identity of nanomaterials to enhance the targeting

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Nanoparticles (NPs) based formulations can enhance the pharmacokinetics & toxicity of conventional drugs, promoting their accumulations in target cells/tissues through the conjugation with different ligands bound on the nano-surface, which mediate the interaction with their receptor. The most common approaches, based on the functionalization with antibodies, peptides or proteins, require a specific control of these biomolecules, ensuring their orientation. However, some issues have been reported using these techniques [1]. Moreover, to reach their destination, NPs are transported through biological fluids, where they can interact with other biomolecules forming the biocorona. This biological coating **(1)** can hide the signal ligand [2], **(2)** can lead to the recognition of the mononuclear phagocyte system (MPS) through biomolecules bound called opsonins [3], **(3)** or proteins absorbed can undergo partial unfolding, triggering a "warning" signal and the later elimination of the NPs [4]. Taking advantage of biotechnology and protein engineering, during my PhD, we proposed a model to customize the identity of the NPs through the conjugation with recombinant ligand-proteins designed with a nanomaterial binding domain, formed by a 6 histidine peptide (**Figure 1**). Our bioconjugation strategy offered a versatile and stable conjugation method that allows protein orientation on the nanomaterial´s surface. Moreover, we got to reduce the binding of the unspecific proteins in the medium (biocorona) [5, 6]. The purpose of the STMS fellowship was to complete the physic-chemical characterization of the system that we had developed using circular dichroism (CD) as a spectroscopy technique to study the protein secondary structure upon interaction with NPs.

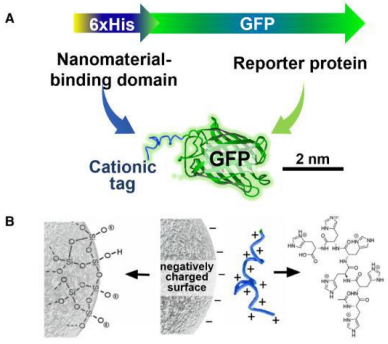

Figure 1.Model proposed. (A) Genetic engineering design. (B) Electrostatic zipper designed between the silica NPand the histidine-tagged protein. (Figure from ref 5).

References

1. Tonigold, M. *et al*. Pre-adsorption of antibodies enables targeting of nanocarriers despite a biomolecular corona. *Nat. Nanotechnol*. 13, 862–869 (2018). 2. Salvati, A. *et al*. Transferrin-functionalized nanoparticles lose their targeting capabilities when a biomolecule corona adsorbs on the surface. *Nat. Nanotechnol*. 8, 137–143 (2013). 3. Gustafson, H. *et al*. Nanoparticle Uptake: The Phagocyte Problem. *Nano Today* 10, 487–510 (2015). 4. Deng, Z. *et al*. Nanoparticle-induced unfolding of fibrinogen promotes Mac-1 receptor activation and inflammation. *Nat. Nanotechnol*. 6, 39–44 (2011). 5. Padín-González, E. *et al.* A custom-made functionalization method to control the biological identity of nanomaterials. *Nanomed.: Nanotechnol. Biol. Med.* 29, 102268 (2020). 6. Navarro-Palomares, E. *et al*. Targeting Nanomaterials to Head and Neck Cancer Cells Using a Fragment of the Shiga Toxin as a Potent Natural Ligand. *Cancers*. *13*, 4920 (2021).

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Computational approach to the study of nano-bio interface

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The latest research in nanoparticle development for anticancer therapy demonstrates their enormous potential for revolutionizing both diagnostic approaches as well as medical treatments. (1,2). The interaction of nanoparticulate systems with biological media, particularly regarding biomolecular corona formation, is of great importance for the successful therapy (3). Computational approach to nano-bio interface can provide a safe, simple and cost-effective way to predict NP behaviour and screen for potential problems in nano-delivery system design. Here, computational models were utilized to determine the events at the interface between metallic nanoparticles and small sulphur-containing biomolecules present in biological media. The surface of silver and gold nanoparticles and their interaction with model molecules – cysteine and glutathione – was described using quantum chemical and molecular dynamics approaches. Computational study using the quantum chemistry software Gaussian 16 included a full conformational search on cysteine, followed by an analysis of binding to Ag and Au clusters. Geometric optimisation, frequency calculations and natural bond orbital calculations of the interaction were performed at the B3LYP/LANL2DZ level of theory. Molecular dynamics calculations were performed in the program package Amber. Cysteine or glutathione were placed in a simulation box with either Ag or Au nanosurfaces, and water molecules. The ligand was situated 15 Å from the surface, and the simulation was run for 70 ns. The obtained trajectories were used to calculate free energies of binding using the MM-GBSA approach. The binding was found to be spontaneous with no desorption, but free lateral diffusion of molecules on metal surfaces. Cysteine and glutathione may bind in various orientations, with all functional groups participating. The analysis on the most stable complexes did not establish the presence of covalent or ionic bonds, but indicated the dipole – induced dipole type of interactions, i.e. the physisorption model. The results of this study contributed to the increase of knowledge about nano-bio interactions and their importance for successful use of nanoparticles in medicine.

References

1. Yang HY, Li Y, Lee DS. Multifunctional and Stimuli-Responsive Magnetic Nanoparticle-Based Delivery Systems for Biomedical Applications. Advanced Therapeutics. 2018; 1:1800011.
2. Singh SK, Singh S, Lillard Jr JW, Singh R. Drug delivery approaches for breast cancer. International Journal of Nanomedicine. 2017; 12:6205-6218.
3. Thanh NTK, Green LAW. Functionalisation of nanoparticles for biomedical applications. Nano Today. 2010; 5:213—230.

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CD-133 functionalized Gold nanoparticles loaded CB839 targeted drug delivery suppressed Glioblastoma stem cells

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The failure treatment of glioblastoma as the most malignant brain tumors in adults can correlate with the presence of glioblastoma stem cells (GSCs) which vastly cause to express some markers on their surface such as CD133. Moreover, extraordinary proliferation of tumor cells are associated with altered metabolic pathway, which leads to increase the rate of glutamine and glucose uptake as the fuel of the cells. Therefore, inhibition of glutaminolysis by utilizing a promising glutaminase1 inhibitor (CB839) can be an effective therapeutic strategy in different types of cancers. In this study, CB839 inhibitor was loaded into a Pegylated Gold nanoparticles (NPs) which covalently bound to CD133 aptamer (AuPEGNPs-CD133-CD839) to evaluate its effect on GSCs including primary and cell lines. GSCs were cultured and treated with different doses of medicine. Our results showed that not only targeted AuPEGNPs-CD839 significantly decreased the viability of glioma cells in a dose-dependent manner, but also it has observed that CD133 conjugated gold nanoparticle can also be effective in some cell lines. To summarize, the data from this study suggest that AuPEGNPs-CD839 have a great potential for suppressing of glioblastoma stem cells, although further studies are required to confirm these achievements and apply to clinic.

References:

1. Giesen B, Nickel AC, Barthel J, Kahlert UD, Janiak C. Augmented Therapeutic Potential of Glutaminase Inhibitor CB839 in Glioblastoma Stem Cells Using Gold Nanoparticle Delivery. Pharmaceutics. 2021 Feb;13(2):295.

2. Peng L, Liang Y, Zhong X, Liang Z, Tian Y, Li S, Liang J, Wang R, Zhong Y, Shi Y, Zhang X. Aptamer-conjugated gold nanoparticles targeting epidermal growth factor receptor variant III for the treatment of glioblastoma. International journal of nanomedicine. 2020;15:1363.

3. Tripathi K, Driskell JD. Quantifying bound and active antibodies conjugated to gold nanoparticles: a comprehensive and robust approach to evaluate immobilization chemistry. ACS omega. 2018 Jul 25;3(7):8253-9.

4. Pan LH, Pang ST, Fang PY, Chuang CK, Yang HW. Label-free biochips for accurate detection of prostate cancer in the clinic: Dual biomarkers and circulating tumor cells. Theranostics. 2017;7(17):4289.

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Iron oxide magnetic nanoparticles modified with biomolecules for theranostic applications

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Iron Oxide Magnetic Nanoparticles (IONPs) attract great interest on the scientific community due to their broad spectrum of applications. As theranostic agents can be used aiming to improve cancer diagnosis and treatment in early stage, reducing simultaneously the side effects on normal tissues (magnetic drug targeting) [1]. One of the most known therapeutic approaches is hyperthermia on the site of tumor by using alternate magnetic field. Furthermore, the IONPs used as contrasts agents (in vivo) in MRI, in gene transformation, biosensors, enzyme immobilization, immunoassays, purification (in vitro) and so on [2].
In the present work we synthesized, full characterized and evaluated the modified carboxylate mNPs that coated first with the co-polymer PEG-co-PLA (mNPs@PEG-co-PLA) through carbodiimide chemistry. The coated mNPs are studied about their loading and release ability of the anticancer drug doxorubicin (DOX). Following the mNPs conjugated with the cyclic Arg-Gly-Asp peptide cRGDfK-Orn3-CGG (mNPs@cRGD) as a targeting peptide through maleimide. Morphological and structural characterization as well as hyperthermia measurements were performed in order to evaluate their heating ability. Both targeted and non- targeted mNPs were radiolabelled with ^{117}Lu performing in-vitro stability as well as in-vivo biodistribution in healthy mice. To investigate the cellular uptake of IONPs we used Prussian Blue assay to identify the mNPs localization and endocytosis mechanism.

References

[1] "Magnetic nanoparticles: synthesis, protection, functionalization, and application.",Lu, A.H., E.L. Salabas, and F. Schuth, Angew Chem Int Ed Engl, 46(8): p. 1222-44, 2007.
[2] "Interaction of metal oxide nanoparticles with lung surfactant protein A." Schulze, C., U.F. Schaefer, C.A. Ruge, W. Wohlleben, and C.M. Lehr, Eur J Pharm Biopharm, 77(3): p. 376-83, 2011

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Drug delivery of doxorubicin with gold nanoparticles

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Nanomedicine is a rapidly evolving field that promises improved therapeutic efficacy and reduced toxicity of medicines. [1] Conjugation of a drug onto gold NPs (AuNPs) may alter the pharmacokinetics and pharmacodynamics and mediate its toxicity. [2] Doxorubicin (DOX), a cytotoxic drug 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 (DOX-AuNPs), and AuNPs just stabilized with PEG (PEG-AuNPs).

Stability assessment showed increase in size and zeta potential for both types of NPs in cell culture medium, which indicated their destabilization. However, this was not observed in the presence of bovine serum albumin (BSA) due to formation of protein corona. Binding affinities were almost similar between BSA and both types of NPs. However, PEGAuNPs caused higher conformational changes in BSA compared to DOXAuNPs. The confocal microscopy images indicated stronger uptake of DOX-AuNPs compared to PEG-AuNPs. Finally, a dose-dependent generation of reactive oxygen species was determined for both NP types, with significantly larger effect shown in PEG-AuNPs.

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

References:

[1] D.D. Gutierrez, M. Surtchev, E. Eiser, C.J. Elsevier, *Nano Lett*., **2006**, *6* 145-147.
[2] S. Rivankar, *J. Cancer Res. Ther*., **2014**, *10*, 853–858.

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Study of imidazolium-terminated carbosilane dendritic systems by EPR

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A new family of imidazolium-terminated carbosilane dendrimers and dendrons was studied as a nanotechnological alternative to current therapies. The aim of this research was the study of the interacting process of nano-structures with a cell membrane model (lecithin liposomes) (see **Figure 1**), with the purpose to use these nano-structures for biomedical applications, *e.g.* as anticancer drug [1]. To carry out this objective, the interactions were measured over time with different generations of dendrimers (G1 to G3) and at increaing concentrations (1, 10 and 20 Mm), using two types of topologies (spheric dendrimers and dendrons). Cell membrane model was studied using computer-aided analysis of electron paramagnetic resonance (EPR) spectra of selected spin probes. The two different paramagnetic probes used (CAT12 and 5SDA) were able to mimiking the phospolipid behavior, providing in this way structural and dynamical information of the membrane in presence of the dendritic systems at different experimental conditions. From this analytical technique it was possible not only to observe that the dendrimer-membrane interaction is generation, topology and concentration dependant, but also the kinetics of internalization, being the fastest at the highest generation. The cytotoxic effect of the dendrimers and the dendrons was verified by an *in vitro* screening in a selection of tumor cell lines, showing a perfect agreement with the EPR results.

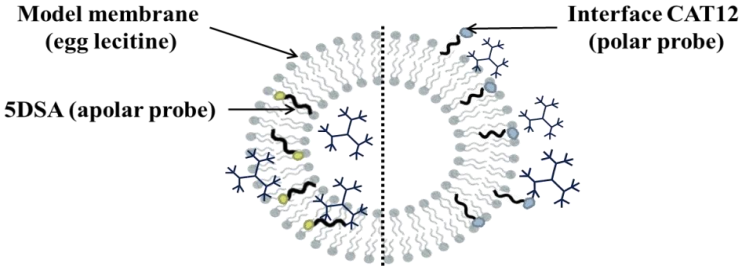

Figure 1. Cell membrane model labelled with a paramagnetic probe in presence of carbosilane dendrimers

References

1. Rodríguez-Prieto, T., Fattori, A., Camejo, C., de la Mata, F. J., Cano, J., Ottaviani, M. F. Gomez, R. Synthesis of imidazolium-terminated carbosilane dendrimers and dendrons and study of their interactions with a cell membrane model, European Polymer Journal. 2020; 133:109748.

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Cationic amino functional bis-MPA based linear-dendritic block copolymers as non-viral vectors of siRNA

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Short interfering RNAs (siRNAs) are being investigated as promising treatments in cancer [1]. However, free siRNA can be easily degraded by ribonucleases (RNases) and recognized by the immune system, causing inflammatory response. Additionally, its poor cellular uptake due to its large size and negative charge is one of the main challenges. Although viral vectors have shown to be the most efficient carriers, there is an increasing interest in developing non-viral vectors due to their higher specificity, modulable synthesis and low immunogenicity. Among all, cationic dendrimers have been in the spotlight as promising non-viral vectors of genetic material due to its nanoscale size as well as high load capacity. Specifically, dendrimers based on 2,2-bis(methylol)propionic acid (bis-MPA) with *beta*-alanine in the periphery showed the ability to successfully transfect glioblastoma cancer cells [2]. The main purpose of this study is to develop an efficient and biocompatible cationic gene vector via structural optimization that can adequately utilize the amine groups for siRNA interaction. This work focuses on the use of dendritic-linear-dendritic (DLD) polymers composed by one hydrophilic chain of polyethylene glycol (PEG) and two bis-MPA dendrons of third and fourth generation functionalized with 16 and 32 cationic *beta*-alanine functional groups at the periphery, respectively. All these systems have proved to form stable nanoconjugates with siRNA at low concentrations at micromolar range and protect it from nucleases degradation. Additionally, one of the main concerns about these cationic carriers is their associated toxicity. Preliminary experiments have shown lack of toxicity of any of these systems in human glioblastoma cancer cells in the range of 0.1-10 micromolar. These results place these derivatives as promising candidates to be evaluated as transfecting agents.

References

1. Chalbatani G M, Dana H, Gharagouzloo E, Grijalvo S, Eritja R, Logsdon C D, Memari F, Miri S R, Rad M R and Marmari V. Small interfering RNAs (siRNAs) in cancer therapy: a nano-based approach. *International Journal of Nanomedicine.* **2019**;14, 3111–3128.
2. Stenström P, Manzanares D, Zhang Y, Ceña V and Malkoch M. Evaluation of Amino-Functional Polyester Dendrimers Based on Bis-MPA as Nonviral Vectors for siRNA Delivery. *Molecules*. **2018**; 23, 2028(1)-2028(15).

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Stability of selenium nanoparticles as novel anticancer delivery vehicle in relevant biological media

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Selenium nanoparticles (SeNPs) represent promising anticancer delivery vehicles due to synergistic effects of the therapeutic cargo, antioxidant and cancer inhibitory activities [1]. Recent studies claim that SeNPs are less toxic than bulk Se forms displaying better biocompatibility and bioefficacy. Moreover, many studies have shown that SeNPs have preventive and therapeutic roles in cancer [2-3]. In order to develop an efficient SeNPs-based drug delivery vehicle, the first step after preparation should be detailed evaluation of the interaction between SeNPs and biological systems, which determines uptake, fate and biological effects of SeNPs.

The aim of this study was to establish and optimize synthetic protocols for two different SeNPs architectures following Safe-by-Design concepts. Full characterization and stability evaluation of these SeNPs was performed in relevant biological media including ultra-pure water, phosphate buffer, cell culture media and blood plasma. The obtained results shown that the complexity of the media, i.e. ionic strength, pH, presence of sugars and proteins have a strong impact on the size distribution, aggregation and surface chemistry of SeNPs. In this way, the first phase for the rationale development of new potential nanotherapeutics was achieved.

Figure 1. Interaction of SeNPs with relevant biological media their effect on surface chemistry of SeNPs.

References

1. Maiyo F, Singh M, Nanomedicine. 2014; 12:1075-1089.
2. Vinković Vrček I. Selenium Nanoparticles: Biomedical Applications. In Selenium. Michalke B (Ed), Springer International Publishing, 2018, 393-413.
3. Zhang Y, Li X, Huang Z, Zheng W, Fan C, ChenT, Nanomedicine. 2013; 9:74-84.

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Synthesis of metal nanoparticles modified with carbosilane dendrons as antitumoral agents

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Gold and silver nanoparticles are considered as a great antitumarol agent, however it has been know that they can cause additional side effect. One of the common side effect caused by metal nanoparticles is effect called NanoEL (endothelial leakage). This effect is undesirable in cancer treatment, because lead to blood leakage in tumor. It has been known that different modification of gold nanoparticles may change their anticancer properties. Therefore, I believe that synthesis and characterization of different type of carbosilane gold (and silver) nanoparticles and further tests connected with blood leakage and creation a holes in endothelial will bring a new knowledge about what type of metal nanoparticles should be prepared to overcome this undesirable side effect.

During STSM PEGylated and dendronized (carbosilane dendrons) metal nanoparticles (Ag was used in this study) were prepared and characterized according to the procedures used in the Prof. J. de la Mata's laboratory. The synthesis and characterization of dendronized AgNPs was divided into several steps. At the first stage, the ready-made silver nanoparticles was used as a base for cationic carbosilane dendrons attachments. Additional modification required the attachment of a PEG chain with the amino terminus at the end. The ratio of attached dendrons to PEG chains was roughly 1 to 1. At this synthesis stage the followed ligands was used: HSG1 (SMe3Cl)2 dendron and two types of polyethylene glycol (PEG)- HS-PEG-OMe and HS-PEG-NH2.The next step was to purifying the synthesized NPs. and determining their concentration. After NP purification was completed, physicochemical analysis was done.

Spectroscopic methods, Dynamic Ligth Scattering, Zeta potential, TEM microscopy and NMR methods were used to characterize obtained nanoparticles. During the analysis, the composition of nanoparticles, the content of individual ligands and the size of the obtained nanoparticles were determined. Obtained NPs will be used in biochemical and cell culture study.

References

1. Andrea Barrios-Gumiel, Javier Sánchez-Nieves, Elzbieta Pedziwiatr-Werbicka, Viktar Abashkin, Natallia Shcharbina, Dzmitry Shcharbin, Sława Glińska, Karol Ciepluch, Dorota Kuc-Ciepluch, Dominika Lach, Maria Bryszewska, Rafael Gómez, F. Javier de la Mata, Effect of PEGylation on the biological properties of cationic carbosilane dendronized gold nanoparticles, International Journal of Pharmaceutics, Volume 573, 2020, 118867

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Development of novel targeted immunomodulatory nanoparticles for colorectal cancer treatment

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Colorectal cancer (CRC) is one of the deadliest cancers worldwide. The severe toxicity of 5-FU regimes renders it unsuccessful with low tumor-specific selectivity. The tumor microenvironment (TME) dictates the treatment outcome and has been proposed to stratify patients with microsatellite unstable (MSI) and stable (MSS) tumors. To provide an effective and targeted therapy, nanomedicine constitute a promising alternative. CEA, an overexpressed molecule in CRC, constitute an interesting candidate to target CRC cells and to modulate the immune response. This work intends to develop an innovative nanosystem to target CEA-expressing CRC cells, carrying the small drug, 5-FU and an immuno strategy, to improve the chemotherapeutic effect on cancer cells. Therefore, the Host Institution has described the development of Nanoemulsion for delivery of RNAs and other nucleic acids, including aptamers. Those strategies will allow us to incorporate immunomodulatory agents capable to trigger the immune system and to modulate CRC's tumor microenvironment. Hence, the expertise of Host Institution fosters the translation from bench to bedside bridging the gap between drug discovery and clinical application, creating news opportunities to improve life's quality of cancer patients. To develop the targeted nanoparticles, chemical conjugation of the polymer with an engineered antibody (scFv) targeting CEA, was performed, followed by the production of the polymeric NPs loaded with 5-FU and Capecitabine. 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. In home institution, the CEA expression in different CRC cell lines was evaluated by flow cytometry and the targeting ability of NPs by immunofluorescence and ImageStream. The NMR spectrum of the conjugated polymer revealed the presence of anti-CEA scFV peaks. Furthermore, 80% of CE was achieved. NPs with 150 nm were attained, with about PdI 0.2 and around 4% of DL. The spherical shape was confirmed. Additionally, Caco-2 was found the cell line with more expression pf CEA and SW480 with no expression. In vitro studies were initiated to assess the binding efficiency and targeting ability of the NPs against CEA-expressing and non-expressing cells confirming its specificity. Therefore, this work demonstrates the successful chemical conjugation of the polymer, with specific targeting of NPs loaded with 5-FU and capecitabine.

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Advanced *in vitro* models for (nano)toxicity determination

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The effort to reduce the use of animal models in preclinical and toxicity tests is closely linked to the development of innovative test systems that will allow better prediction of the *in vivo* response.
The technology of microfluidic systems has rapidly grown in recent decades and has been widely used in several scientific disciplines, notably due to miniaturization, increased sensitivity, and better recapitulation of physiological conditions [1]. Microfluidic systems coupled with the use of different cell types offer an alternative platform, for example, for predicting the efficacy, toxicity, and pharmacokinetics of new drugs or as an innovative approach to screening for the effects of xenobiotics [2]. Such advanced *in vitro* systems aim, in addition to standard testing, to reduce the need for animal testing with the ambition to replace conventional screening techniques [3].
Under *in vivo* conditions, the liver is the major organ in which xenobiotics are metabolized and transformed, and the kidneys are subsequently responsible for their uptake, concentration, and elimination from the body. Thus, both organs play a key role in the detoxification and elimination of xenobiotics and their metabolites and represent the two primary targets of the toxic effect of xenobiotics (including drugs and nanoparticles) [4]. Studies using co-culture of liver-kidney cells occur sporadically in the literature and by selecting appropriate model systems, this arrangement could provide valuable information on the interaction between tissues, as well as reliable and effective prediction of the toxic effects of xenobiotics.

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References

1. Coluccio M.L., Perozziello G., Malara N., Parrotta E., Zhang P., Gentile F., Limongi T., Raj P.M., Cuda G., Candeloro P., Di Fabrizio E. Microfluidic platforms for cell cultures and investigations. Microelectronic Engineering. 2019; 208: 14–28.
2. Cui P. and Wang S. Application of microfluidic chip technology in pharmaceutical analysis: A review. Journal of Pharmaceutical Analysis 2019; 9: 238–247.
3. Tehranirokh M., Kouzani1 A.Z., Francis P.S., Kanwar J.R. Microfluidic devices for cell cultivation and proliferation. Biomicrofluidics 2013; 7: 051502.
4. Bajaj P., Chowdhury S.K.,Yucha R., Kelly E.J., Xiao G. Emerging Kidney Models to Investigate Metabolism, Transport, and Toxicity of Drugs and Xenobiotics. Drug Metab Dispos 2018; 46: 1692–1702.

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Glucose-decorated Carbosilane dendrimers interacting with a model cell membrane, studied by means of the spin-probe EPR technique

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The chemicals which are constructed to be used as a drug delivery systems (DDS) have to be deeply investigated in the area of their influence on biological structures. Results obtained by monitoring of DDS interactions with model lipid membranes can help to understand their mechanism of toxicity by influencing membrane biophysical properties and/or integrity [1–3].

To investigate dendrimers-lipid membrane interactions, liposomes created from negatively charged mixture of phosphatidylcholines (LEC) by extrusion method were used. Samples were investigated by EPR method with two spin probes CAT12 and 5DSA. The final lipid concentration in the samples was 20 mM, the concentrations of dendrimers were 1 and 2 mM and the phospholipid/probe molar ratio was 20:1. For all samples, order and microviscosity decrease over the incubation time, even if in the first hours the decrease was slower, mainly at the lower generations. With CAT12 order and microviscosity increase with the increase in generation, as also found with 5DSA, being the highest the G3 ones. The main differences between the 5DSA and CAT12 results are that (a) CAT12+LEC system in the absence of dendrimers shows higher microviscosity and order than in the presence of dendrimers, while the opposite holds for 5DSA. Therefore the dendrimers, interacting with the liposomes, provoke an increased packing of the liposome structure in the lipidic region, while they perturb the liposome structure at the interface, where the CAT12 group is placed; (b) the liposome-structure perturbation played by the dendrimers, mainly G1 and G2, occurred at the beginning, while at the later hours the microviscosity and order values go to converge. Conversely, the structural packing sensed by 5DSA mainly occurred at the later hours. EPR analysis provides useful information about the interactions occurring between the LEC liposomes and the dendrimers, allowing to verify how these interactions change in type and strength with generation and dendrimer concentration, as such as over the incubation time.

References

[1] S. Hong, A.U. Bielinska, A. Mecke, B. Keszler, J.L. Beals, X. Shi, L. Balogh, B.G. Orr, J.R. Baker, Interaction of Poly (amidoamine) Dendrimers with Supported Lipid Bilayers and Cells : Hole Formation and the Relation to Transport, Bioconjug. Chem. 15 (2004) 774–782.
[2] D. Wrobel, D. Appelhans, M. Signorelli, B. Wiesner, D. Fessas, U. Scheler, B. Voit, J. Maly, Interaction study between maltose-modified PPI dendrimers and lipidic model membranes, Biochim. Biophys. Acta - Biomembr. 1848 (2015) 1490–1501.
[3] B. Klajnert, J. Janiszewska, Z. Urbanczyk-Lipkowska, DSC studies on interactions between low molecular mass peptide dendrimers and model lipid membranes, Int. J. Pharm. 327 (2006) 145–152.

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Anti-inflammatory properties of carbosilane dendrimers

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Long term inflammation is associated with several inflammation-associated diseases, such as asthma, multiple sclerosis, rheumatoid arthritis and cancer [1]. To prevent the development of diseases associated with chronic inflammation condition inflammatory response has to be controlled and all chemicals used for medical treatment have to be checked in the area of their pro-inflammatory properties. Four types of model systems were created: i) THP-1 cells activated by LPS and incubated with dendrimers for 3 h, ii) THP-1 cells incubated with dendrimers for 3h, iii) THP-1 cells activated by LPS, incubated with dendrimers for 3 h and incubated in new medium for 21h and iv) THP-1 cells incubated with dendrimers for 3 h and incubated in new medium for 21h. After incubation procedures, mRNA was extracted, complementary DNA was synthesized and samples were investigated by RT-PCR technique. The changes in a activation level of four genes contributed to inflammatory response NFKBIA, BTG2, IL1B, TNF were investigated as well as three reference genes: TBP, HMBS and hPRB. After 3h incubation carbosilane dendrimers mainly with hydrophobic functional groups were able to increased expression of NF-κB marker genes (NFKBIA and BTG2). Interleukin 1β and TNFα genes were also activated by all investigated carbosilane dendrimers. Fortunately, in case of cells pre-activated with LPS over expression of pro-inflammatory genes was observed at the level of control. Dendrimers with hydroxyl functional groups induced a positive synergistic effect for all four genes. Positive synergistic effect of gene activation was also detected for IL1 for phosphonium carbosilane dendrimers decorated with phenyl and metoxyphenyl groups. Cells incubated all together for 24h one part incubated 3h with dendrimers and 21h in fresh medium, second part activated with LPS, incubated 3h with dendrimers and 21h in a fresh medium. Not treated with LPS cells showed lack of gene activation process on TNFα, NFKBIA and BTG2 genes for almost all dendrimers - only dendrimers with hydroxyl functional groups very strong activating all genes. Cells activated with LPS and incubated 24 hours positive synergistic effect of inflammatory activation was observed for butyl and phenyl terminated phosphonium carbosilane dendrimers. Whereas, protection effect was observed for ammonium carbosilane dendrimers and phosphonium metoxyphenyl terminated carbosilane dendrimers. Positive synergistic effect after 24h incubation of NFKBIA gene activation was observed for all investigated dendrimers (the strongest effect was observed for phenyl terminated). Comparing all results the best anty-inflammatory profile was shown for phosphonium metoxyphenyl terminated carbosilane dendrimers. This effect was visible the best for cells activated with LPS and incubated for 24h.

References

1. Kunnumakkara, A.B., Sailo, B.L., Banik, K. *et al.* Chronic diseases, inflammation, and spices: how are they linked?. *J Transl Med* **16** (2018) 1-25

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