ABSTRACTS OF THE SCIENTIFIC PAPERS

Of senior assistant professor **Kameliya Todorova Hristova-Panusheva,**
Candidate for participation in the competition
for the academic position „ASSOCIATE PROFESSOR“
field of higher education 4. Natural Sciences, Mathematics and Informatics,
professional field 4.3. Biological Sciences, scientific specialty “Biophysics”
to the Department “Electroinduced and Adhesive Properties”,
Institute of Biophysics and Biomedical Engineering,
Bulgarian Academy of Sciences,
announced in SG issue 58/18.07.2025

Publications in indicator group B

Indicator B4: Scientific publications in journals referenced and indexed in a world-renowned database of scientific information (Web of Science и Scopus)

B4.1. Poornima Budime Santhosh , **Kamelia Hristova-Panusheva**, Todor Petrov, Lyubomir Stoychev, Natalia Krasteva and Julia Genova, Femtosecond Laser-Induced Photothermal Effects of Ultrasmall Plasmonic Gold Nanoparticles on the Viability of Human Hepatocellular Carcinoma HepG2 Cells, *Cells, 2024, 13(24), 2139,* ISSN 20734409, DOI 10.3390/cells13242139, **IF 2024 5.1 Q1 (Scopus) 25 p.**
https://www.mdpi.com/2073-4409/13/24/2139

Abstract: Laser-induced photothermal therapy using gold nanoparticles (AuNPs) has emerged as a promising approach to cancer therapy. However, optimizing various laser parameters is critical for enhancing the photothermal conversion efficacy of plasmonic nanomaterials. In this regard, the present study investigates the photothermal effects of dodecanethiol-stabilized hydrophobic ultrasmall spherical AuNPs (TEM size 2.2 ± 1.1 nm), induced by a 343 nm wavelength ultrafast femtosecond-pulse laser with a low intensity (0.1 W/cm^2) for 5 and 10 min, on the cell morphology and viability of human hepatocellular carcinoma (HepG2) cells treated in vitro. The optical microscopy images showed considerable alteration in the overall morphology of the cells treated with AuNPs and irradiated with laser light. Infrared thermometer measurements showed that the temperature of the cell medium treated with AuNPs and exposed to the laser increased steadily from 22 °C to 46 °C and 48.5 °C after 5 and 10 min, respectively. The WST-1 assay results showed a significant reduction in cell viability, demonstrating a synergistic therapeutic effect of the femtosecond laser and AuNPs on HepG2 cells. The obtained results pave the way to design a less expensive, effective, and minimally invasive photothermal approach to treat cancers with reduced side effects.

B4.2. Charilaos Xenodochidis; **Kamelia Hristova-Panusheva**; Trayana Kamenska; Poornima Budime Santhosh; Todor Petrov; Lyubomir Stoychev; Julia Genova; Natalia Krasteva, Graphene Oxide Nanoparticles for Photothermal Treatment of Hepatocellular Carcinoma Using Low-Intensity Femtosecond Laser Irradiation, *Molecules*, 2024, 29(23), 5650, ISSN 14203049, DOI 10.3390/molecules29235650, **IF 2024 4.2, Q1** (Scopus) **25 p.**
https://www.mdpi.com/1420-3049/29/23/5650

Abstract: Graphene oxide-mediated photothermal therapy using femtosecond lasers has recently shown promise in treating hepatocellular carcinoma. However, significant work

remains to optimize irradiation parameters for specific nanoparticle types and cancer cells to improve nanomaterial-mediated photothermal anticancer therapy. This study investigated the photothermal potential of nGO and nGO-PEG nanoparticles (NPs) combined with femtosecond laser irradiation at 515 nm and 1030 nm wavelengths, with varying power (0.1 and 0.2 W/cm^2) and duration (5 and 10 min), to optimize photothermal therapy for hepatocellular carcinoma. Conversion efficiency of NPs, morphology and viability of HepG2 and normal MDCK cells after treatments were evaluated using an electronic thermometer, phase-contrast microscopy, and WST-1 assay. The results revealed that nGO-PEG NPs exhibited better photothermal efficiency than nGO, with 515 nm of irradiation inducing a temperature increase up to 19.1 °C compared to 4.7 °C with 1030 nm of light. Laser exposure to 515 nm significantly reduced HepG2 cell viability, with the most intense conditions (10 min at 0.2 W/cm^2) causing a decrease of up to 58.2% with nGO and 43.51% with nGO-PEG. Normal MDCK cells showed minimal impact or a slight viability increase, especially with nGO-PEG. Combined treatment with laser irradiation and NPs induced significant morphological changes in HepG2 cells, including cell detachment and apoptotic-like characteristics, particularly with 1030 nm of irradiation. MDCK cells exhibited minimal morphological changes, with some recovery observed under lower energy conditions. These findings suggest that low-energy lasers and engineered nanomaterials could provide a minimally invasive approach to photothermal cancer therapy with reduced side effects.

B4.3. Hristova-Panusheva, K., Xenodochidis, C., Georgieva, M., Krasteva, N. Nanoparticle-Mediated Drug Delivery Systems for Precision Targeting in Oncology*, Pharmaceuticals*, 2024, 17(6), 677, ISSN 14248247, DOI 10.3390/ph17060677, **IF$_{2024}$ 4.3 Q2 20 p.**
https://www.mdpi.com/1424-8247/17/6/677

Abstract: Nanotechnology has emerged as a transformative force in oncology, facilitating advancements in site-specific cancer therapy and personalized oncomedicine. The development of nanomedicines explicitly targeted to cancer cells represents a pivotal breakthrough, allowing the development of precise interventions. These cancer-cell-targeted nanomedicines operate within the intricate milieu of the tumour microenvironment, further enhancing their therapeutic efficacy. This comprehensive review provides a contemporary perspective on precision cancer medicine and underscores the critical role of nanotechnology in advancing site-specific cancer therapy and personalized oncomedicine. It explores the categorization of nanoparticle types, distinguishing between organic and inorganic variants, and examines their significance in the targeted delivery of anticancer drugs. Current insights into the strategies for developing actively targeted nanomedicines across various cancer types are also provided, thus addressing relevant challenges associated with drug delivery barriers. Promising future directions in personalized cancer nanomedicine approaches are delivered, emphasising the imperative for continued optimization of nanocarriers in precision cancer medicine. The discussion underscores translational research's need to enhance cancer patients' outcomes by refining nanocarrier technologies in nanotechnology-driven, site-specific cancer therapy.

B4.4. Milena Keremidarska-Markova; Iliyana Sazdova; Bilyana Ilieva; Milena Mishonova; Milena Shkodrova; **Kamelia Hristova-Panusheva**; Natalia Krasteva; Mariela Chichova, Comprehensive Assessment of Graphene Oxide Nanoparticles: Effects on Liver Enzymes and Cardiovascular System in Animal Models and Skeletal Muscle Cells, *Nanomaterials*, 2024, 14(2), 188, ISSN 20794991, DOI 10.3390/nano14020188, **IF $_{2024}$ 4.4 Q1** 25 p. (Scopus)
https://www.mdpi.com/2079-4991/14/2/188

Abstract: The growing interest in graphene oxide (GO) for different biomedical applications requires thoroughly examining its safety. Therefore, there is an urgent need for reliable data on how GO nanoparticles affect healthy cells and organs. In the current work, we adopted a comprehensive approach to assess the influence of GO and its polyethylene glycol-modified form (GO-PEG) under near-infrared (NIR) exposure on several biological aspects. We evaluated the contractility of isolated frog hearts, the activity of two rat liver enzymes–mitochondrial ATPase and diamine oxidase (DAO), and the production of reactive oxygen species (ROS) in C2C12 skeletal muscle cells following direct exposure to GO nanoparticles. The aim was to study the influence of GO nanoparticles at multiple levels - organ; cellular; and subcellular - to provide a broader understanding of their effects. Our data demonstrated that GO and GO-PEG negatively affect heart contractility in frogs, inducing stronger arrhythmic contractions. They increased ROS production in C2C12 myoblasts, whose effects diminished after NIR irradiation. Both nanoparticles in the rat liver significantly stimulated DAO activity, with amplification of this effect after NIR irradiation. GO did not uncouple intact rat liver mitochondria but caused a concentration-dependent decline in ATPase activity in freeze/thaw mitochondria. This multifaceted investigation provides crucial insights into GOs potential for diverse implications in biological systems.

B4.5. Gospodinova, Z., **Hristova-Panusheva, K.,** Kamenska, T. *et al.* Insights into cellular and molecular mechanisms of graphene oxide nanoparticles in photothermal therapy for hepatocellular carcinoma. *Sci Rep* 15, 15541 (2025). **IF 2024 3.9, Q1 25p**
https://doi.org/10.1038/s41598-025-99317-w

Abstract: Graphene oxide derivatives have shown promise for photothermal cancer therapy due to their efficient light-to-heat conversion in the near-infrared (NIR) range. Therefore, in this study, we investigated the potential of newly synthesized pristine (nGO) and PEGylated (nGO-PEG) graphene oxide nanoparticles, for photothermal therapy of hepatocellular carcinoma (HepG2) cells. We evaluated various aspects of cellular behavior, including migration, growth, morphology, cell membranes integrity, mitochondrial dynamics, actin cytoskeleton organization, and ROS generation along with the expression of genes linked to apoptosis (*CASP8*, *BAX*), autophagy (*BECN1*), cell cycle arrest (*CDKN1A*), and metastasis (*HMMR*). Our findings reveal that 5 min of 808 nm NIR irradiation caused a mild temperature increase enhancing cytotoxicity, with nGO showing higher toxicity by disrupting cell morphology, reducing proliferation, and increasing ROS levels. In contrast, nGO-PEG more effectively suppressed cell motility and demonstrated improved biocompatibility. Gene expression analysis revealed upregulation of apoptosis-related genes in nGO-PEG-treated cells indicating mitochondrial damage, while nGO induced autophagy, as seen by increased *BECN1* expression. The findings point to distinct therapeutic potentials: nGO as a potent cytotoxic agent inducing autophagy, and nGO-PEG as a more biocompatible nanoparticle promoting apoptosis. This dual-pathway analysis provides a basis for tailored therapeutic strategies for liver cancer.

Publications in indicator group G

Indicator G 7 Abstracts of scientific publications in English, in journals refereed and indexed in world-recognized databases for scientific information (Web of Science and Scopus)

G7.1. Valtcheva-Sarker, Ralitca, Stephanova, Elena, **Hristova, Kamelia**, Altankov, George, Momchilova, Albena, Pankov, Roumen, Halothane affects focal adhesion proteins in the A 549 cells, *Molecular and Cellular Biochemistry*, 2007, 295(1-2), pp. 59–64, ISSN 15734919, DOI10.1007/s11010-006-9272-x, **IF** 2007 **1.707, Q1 25 p.**
https://link.springer.com/article/10.1007/s11010-006-9272-x

Abstract: Halothane is a volatile anaesthetic, which is known to induce alterations in cellular plasma membranes, modulating the physical state of the membrane lipids and/or interacting directly with membrane-bound proteins, such as integrin receptors. Integrin-mediated cell adhesion is a general property of eukaryotic cells, which is closely related to cell viability. Our previous investigations showed that halothane is toxic for A 549 lung carcinoma cells when applied at physiologically relevant concentrations and causes inhibition of adhesion to collagen IV. The present study is focused on the mechanisms underlying halothane toxicity. Our results imply that physiologically relevant concentrations of halothane disrupt focal adhesion contacts in A 549 cells, which is accompanied with suppression of focal adhesion kinase activity and paxillin phosphorylation, and not with proteolytic changes or inhibition of vinculin and paxillin expression. We suggest that at least one of the toxic effects of halothane is due to a decreased phosphorylation of the focal contact proteins.

G7.2. N A Krasteva, G Toromanov, **K T Hristova**, E I Radeva, E V Pecheva, R P Dimitrova, G P Altankov and L D Pramatarova, Initial biocompatibility of plasma polymerized hexamethyldisiloxane films with different wettability, *Journal of Physics: Conference Series,* 2010, 253(1), 012079, DOI 10.1088/1742-6596/253/1/012079, **SJR** 2010 **0.288 10 p.**
https://iopscience.iop.org/article/10.1088/1742-6596/253/1/012079

Abstract: Understanding the relationships between material surface properties, behaviour of adsorbed proteins and cellular responses is essential to design optimal material surfaces for tissue engineering. In this study we modify thin layers of plasma polymerized hexamethyldisiloxane (PPHMDS) by ammonia treatment in order to increase surface wettability and the corresponding biological response. The physico-chemical properties of the polymer films were characterized by contact angle (CA) measurements and Fourier Transform Infrared Spectroscopy (FTIR) analysis.Human umbilical vein endothelial cells (HUVEC) were used as model system for the initial biocompatibility studies following their behavior upon preadsorption of polymer films with three adhesive proteins: fibronectin (FN), fibrinogen (FG) and vitronectin (VN). Adhesive interaction of HUVEC was evaluated after 2 hours by analyzing the overall cell morphology, and the organization of focal adhesion contacts and actin cytoskeleton. We have found similar good cellular response on FN and FG coated polymer films, with better pronounced vinculin expression on FN samples while. Conversely, on VN coated surfaces the wettability influenced significantly initial celular interaction spreading. The results obtained suggested that ammonia plasma treatment can modulate the biological activity of the adsorbed protein s on PPHMDS surfaces and thus to influence the interaction with endothelial cells.

G7.3. L D Pramatarova, N A Krasteva, E I Radeva, E V Pecheva, R P Dimitrova, T A Hikov, D P Mitev, **K T Hristova** and G Altankov, Study of detonation nanodiamond - Plasma polymerized hexamethildisiloxan composites for medical application, *Journal of Physics: Conference Series*, 2010, 253(1), 012078, DOI 10.1088/1742-6596/253/1/012078, **SJR** 2010**0.288 10 p.**
https://iopscience.iop.org/article/10.1088/1742-6596/253/1/012078

Abstract: The present study reports on how detonation nanodiamond (DND) - plasma poly(hexamethyldisiloxane) composites (PPHMDS) affect osteoblast cell behavior. It has been established that various modified DND nanoparticles (Ag-DND and Si-DND) can be readily integrated into virtually all polymer matrices. In particular, PPHDMS composites have been developed over the past few years because of the variety of their application as medical devices and implants. By incubation of MG-63 osteoblast-like cells on the surface of DND (Ag-DND and Si-DND) - PPHMDS composite, we tested the hypothesis that DND–based polymer composites can influence the adhesion behavior of MG-63 osteoblast-like cells. Morphological and structural characterization of DND, Ag-DND and Si-DND powders was carried out by XRD, HRTEM and EDS. For the study of the composite layers, deposited on cover glass (CG), FTIR spectroscopy has been performed in order to determine if the DND nanofiller can potentially modify the structural and chemical dynamics of the polymer matrix. The kinetic of static water contact angle of composite surfaces as a function of the as-used nanofiller DND's in polymer matrix was measured The results with MG-63 osteoblast-like cells suggest the potential of using DND-based polymer composites for application in engineering implantable scaffolds and devices.

G7.4. N. Krasteva; **K. Hristova**; E. Radeva; E. Pecheva; R. Dimitrova; L. Pramatarova, Effect of ammonia plasma treatment on the biological performance of plasma polymerized hexamethyldisiloxane, *AIP Conf. Proc.* 1203, 688–693 (2010), DOI 10.1063/1.3322536, **SJR 2010 0.166 10 p.**
https://pubs.aip.org/aip/acp/article-abstract/1203/1/688/928598/Effect-of-Ammonia-Plasma-Treatment-on-the?redirectedFrom=fulltext

Abstract: Plasma polymerized hexamethyldisiloxane (PPHMDS) is a polymer frequently considered for biomedical application due to its easy fabrication, low cost and biocompatibility. However, a drawback of using PPHMDS in osteosubstitutive engineering is its high hydrophobicity, causing extremely low cellular interaction. Surface wettability can be altered in many ways such as plasma treatment, surface coating, introduction of hydrophilic or hydrophobic groups by self-assembled monolayers, etc. Among all these methods plasma treatment is one of most effective and economical ways to modify surface wettability of a material. Here, we investigated three different PPHMDS layers prepared by plasma polymerization (PP) of HMDS on cover glass (CG) under two different technological regimes. In order to improve cellular interactions, PPHMDS films obtained by the second operating mode were further hydrophylized in NH3 plasma. Cell-biomaterial interactions were characterized by human osteoblast-like MG63 cell model, where overall cell morphology, viability, proliferation and alkaline phosphatase activity were measured after 1, 3, 7 and 10 days of cultivation. The results emphasize a potential of ammonia plasma treatment for polymer hydrophilization and improved osteoblast-material interactions in order to use them in bone tissue engineering.

G7.5. Keremidarska-Markova, M., **Hristova-Panusheva, K.**, Vladkova, T., Krasteva, N., Adipose-derived mesenchymal stem cell behaviour on PDMS substrates with different

hardness, *Comptes Rendus de L'Academie Bulgare des Sciences,* 2017, 70(5), pp. 663–670, ISSN 13101331, **IF** $_{2017}$ **0.270 Q2 20 p.**
https://www.scopus.com/record/display.uri?eid=2-s2.0-85019770637&origin=recordpage

Abstract: Optimising the properties of materials for tissue engineering applications needs a comprehensive understanding of the behaviour and responses of cells cultured on these materials. Recently, substrate mechanical properties, in addition to biochemical signals, have been shown to be a sensitive regulator of stem cell behaviour. The aim of this work was to investigate the effects of bulk hardness of polydimethylsiloxane (PDMS) materials on human adipose-derived mesenchymal stem cells (hAD-MSCs) behaviour and to find correlation with material surface properties. For this, PDMS substrates with different Shore hardness (40, 55 and 70) were prepared and characterized in respect to their surface properties and efficiency of fibronectin matrix secretion, cell proliferation and osteogenic differentiation. Surface properties of PDMS substrates were found to alter non-linearly with increasing the bulk hardness. FN synthesis and osteogenic differentiation were increased on PDMS substrates with intermediate bulk hardness (55) while cell proliferation decreased with increasing of PDMS hardness. These findings suggest that the effect of substrate hardness on stem cell behaviour is complicated and it is tightly connected to the material's surface properties that also should be taken in account into development of biomaterials for bone tissue engineering.

G7.6. Keremidarska-Markova, M.; Radeva, E.; Mitev, D.; **Hristova-Panusheva, K**.; Paull, B.; Nesterenko, P.; Šepitka, J.; Junkar, I.; Iglič, A.; Krasteva, N., Increased elastic modulus of plasma polymer coatings reinforced with detonation nanodiamond particles improves osteogenic differentiation of mesenchymal stem cells, *Turkish Journal of Biology*, 2018, 42(2), pp. 195–203, ISSN 13000152, DOI 10.3906/biy-1711-26, **SJR** $_{2018}$ **0.268 Q3 15 p.**
https://journals.tubitak.gov.tr/biology/vol42/iss2/10/

Abstract: In the present study we demonstrated that composite PPHMDS/DND coatings with elastic moduli close to those of mature bone tissue (0.2-2.8 GPa) stimulated growth and osteogenic differentiation of human adipose-derived mesenchymal stem cells (hAD-MSCs). Composite coatings were prepared by a method of plasma polymerization (PP) where detonation nanodiamond (DND) particles in different amounts (0.1, 0.5, and 1 mg/mL) were added to hexamethyldisiloxane (HMDS) before plasma deposition. This method allows variation only in the reduced elastic modulus (Er') with increase in the particle concentration, while the other surface properties, including surface wettability and topography, did not change. The response of hAD-MSCs to the increasing stiffness showed an effect on adhesion and osteogenic differentiation but not on cell proliferation. Matrix mineralization and cell spreading were maximized on PPHMDS/DND coatings with the highest elastic modulus (2.826 GPa), while the differences in proliferation rates among the samples were negligible. In general, PPHMDS/DND coatings provide better conditions for growth and osteogenic differentiation of hAD-MSCs in comparison to glass coverslips, confirming their suitability for osteo-integration applications. Additionally, our findings support the hypothesis that biomaterials with elasticity similar to that of the native tissue can improve the differentiation potential of mesenchymal stem cells.

G7.7. Milena Keremidarska-Markova; **Kamelia Hristova-Panusheva**; Tonya Andreeva; Giorgio Speranza; Dayong Wang; Natalia Krasteva, Cytotoxicity Evaluation of Ammonia-Modified Graphene Oxide Particles in Lung Cancer Cells and Embryonic Stem Cells,

Advances in Condensed Matter Physics, 2018, 9571828, ISSN 16878108, DOI 10.1155/2018/9571828, **SJR 2018 0.289 Q3 15 p.**
https://onlinelibrary.wiley.com/doi/10.1155/2018/9571828

Abstract: Potential toxicity of graphene oxide (GO) is a subject of increasing research interest in the recent years. Here, we have evaluated the cytotoxicity of ammonia-modified GO (GO-NH_2) and pristine GO particles in human lung cancer cells, A549 and embryonic stem cells, Lep3 exposed to different particles concentrations (0.1, 1, 10, 20, and 50 μg/ml) for different times (24 and 48h). Compared with GO, GO-NH_2 particles possessed smaller size, positive surface charge and higher thickness. An increased propensity to aggregation in cell cultures was also found for GO-NH_2 particles. Cytotoxicity evaluation revealed that GO-NH_2 particles are more toxic than pristine GO. Applied at concentrations of 10, 20 and 50 μg/ml for 24h they affect significantly cell morphology of viable embryonic stem cells whereas human lung cancer A549 cells seem to be relatively more resistant to short-time exposure. After 48h exposure however cell proliferation of A549 cells was strongly suppressed in a dose-dependent manner while the proliferation ability of embryonic stem cells was not affected. These results suggested that both GO particles exert different degree of cytotoxicity which is time, dose and cell dependent. In general, ammonia-modified GO particles are more toxic than the pristine GO which should be taken into account for future biomedical applications.

G7.8. Hristova-Panusheva, K., Keremidarska-Markova, M.; Andreeva, T., Speranza, G., Wang, D., Georgieva, M., Miloshev, G., Krasteva, N., Dose-dependent genotoxicity of ammonia-modified graphene oxide particles in lung cancer cells, *Journal of Physics: Conference Series,* 2019, 1186(1), 012009, ISSN 17426588, DOI 10.1088/1742-6596/1186/1/012009, **SJR 2019 0.227 10 p.**
https://iopscience.iop.org/article/10.1088/1742-6596/1186/1/012009

Abstract: Graphene oxide (GO), the water soluble form of 2D graphene, has received much attention because of its attractive properties for a wide range of applications and products. Surface modification with different functional groups can improve GO biocompatibility for further biomedical applications. In the present study we have evaluated genotoxicity of pristine and ammonia-modified graphene oxide (GO-NH_2) nanoparticles (NPs) in a human lung epithelial cell line, A549, exposed for 24 h to different concentrations of NPs (0.1, 1, 10, 20 and 50 μg/ml). Quantification of reactive oxygen species (ROS) indicated that exposure to higher concentrations of both types of NPs resulted in enhanced ROS generation. The observed comet tail migration in the method of Single Cell Gel Electrophoresis in the cells treated with 20 and 50 μg/ml GO and GO-NH_2 indicated presence of damages in DNA. Cell cycle analysis showed that after treatment of A549 cells with increasing concentrations of NPs for 24h the percentage of cells in G0/G1 phase of the cell cycle decreased while the percentage of cells in G2/M increased. The presented results suggest that ammonia-modified GO NPs applied at concentrations higher than 20 μg/ml induced stronger toxicity effect in A549 cells compared to pristine GO and that the use of low concentrations of GO and GO-NH_2 NPs is important to avoide adverse biological effects.

G 7.9. Natalia Krasteva, Milena Keremidarska-Markova, **Kamelia Hristova-Panusheva**, Tonya Andreeva, Giorgio Speranza, Dayong Wang, Milena Draganova-Filipova, George Miloshev, Milena Georgieva, Aminated Graphene Oxide as a Potential New Therapy for Colorectal Cancer, *Oxidative Medicine and Cellular Longevity*, Vol 2019, 2019, 3738980,

ISSN 19420900, DOI 10.1155/2019/3738980, **IF 2024 5.07 Q1 25 p.**
https://onlinelibrary.wiley.com/doi/10.1155/2019/3738980

Abstract: Nanotechnology-based drug delivery systems for cancer therapy are the topic of interest for many researchers and scientists. Graphene oxide (GO) and its derivates are among the most extensively studied delivery systems of this type. The increased surface area, elevated loading capacity, and aptitude for surface functionalization together with the ability to induce reactive oxygen species make GO a promising tool for the development of novel anticancer therapies. Moreover, GO nanoparticles not only function as effective drug carriers but also have the potential to exert their own inhibitory effects on tumour cells. Recent results show that the functionalization of GO with different functional groups, namely, with amine groups, leads to increased reactivity of the nanoparticles. The last steers different hypotheses for the mechanisms through which this functionalization of GO could potentially lead to improved anticancer capacity. In this research, we have evaluated the potential of amine-functionalized graphene oxide nanoparticles (GO-NH_2) as new molecules for colorectal cancer therapy. For the purpose, we have assessed the impact of aminated graphene oxide (GO) sheets on the viability of colon cancer cells, their potential to generate ROS, and their potential to influence cellular proliferation and survival. In order to elucidate their mechanism of action on the cellular systems, we have probed their genotoxic and cytostatic properties and compared them to pristine GO. Our results revealed that both GO samples (pristine and aminated) were composed of few-layer sheets with different particle sizes, zeta potential, and surface characteristics. Furthermore, we have detected increased cyto- and genotoxicity of the aminated GO nanoparticles following 24-hour exposure on Colon 26 cells. The last leads us to conclude that exposure of cancer cells to GO, namely, aminated GO, can significantly contribute to cancer cell killing by enhancing the cytotoxicity effect exerted through the induction of ROS, subsequent DNA damage, and apoptosis.

G7.10. Hikov, T., Krasteva, N., **Hristova-Panusheva, K.**, Ivanov, N., Petrov, P., Study on the biocompatibility of TiN/TiO 2 bilayer coatings deposited by DC magnetron sputtering on stainless steel, *AIP Conference Proceedings*, 2019, 2075, 160022, ISBN 978-073541803-5, DOI 10.1063/1.5091349, **SJR 2019 0.19 10 p.**
https://pubs.aip.org/aip/acp/article-abstract/2075/1/160022/695677/Study-on-the-biocompatibility-of-TiN-TiO2-bilayer?redirectedFrom=fulltext

Abstract: Multilayer coatings such as TiN/TiO2 are widely used in modern medicine and dentistry. They improve the mechanical properties of the substrate and its biocompatibility. The substrates can be different kind of metals, alloys which are used for manufacturing implants, rotary dental instruments, endodontic instruments, joints, etc. It is expected that materials will be universally accepted and will not cause harm or injury to the surrounding structures. Therefore, in this study, TiN/TiO2 multilayer coatings were deposited on stainless steel 304 by DC magnetron sputtering. The structure of the coatings was observed by XRD (X-ray diffraction) with Cu Kα characteristic radiation (1.54 Å). The measurements were conducted in Bragg-Brentano (B-B) symmetrical mode, from 20° to 80° at 2θ scale. The step has been chosen 0.1° with counting time 10 sec. per step. The biocompatibility of the coatings was studied by CCK-8 assay of osteoblastic MG63 cells, incubated for 72 hours on the samples. Our results showed that the obtained TiN/TiO2 multilayer coatings have stoichiometries and don't suppress cell growth and spreading which means that the coatings are not cytotoxic and they are biocompatible.

G7.11. Świerczek-Lasek, B.; Keremidarska-Markova, M.; **Hristova-Panusheva, K.**; Vladkova, T.; Ciemerych, M.A.; Archacka, K.; Krasteva, N., Polydimethylsiloxane materials with supraphysiological elasticity enable differentiation of myogenic cells, *Journal of Biomedical Materials Research - Part A*, 2019, 107(12), pp. 2619–2628, ISSN 15493296, DOI 10.1002/jbm.a.36768, **IF 2019 3.525 Q1 25 p**.
https://www.scopus.com/record/display.uri?eid=2-s2.0-85070742212&origin=recordpage

Abstract: Myogenic differentiation during muscle regeneration is guided by various physical and biochemical factors. Recently, substratum elasticity has gained attention as a physical signal that influences both cell differentiation and tissue regeneration. In this work, we investigated the influence of substratum elasticity on proliferation and differentiation of myogenic cells, mouse myoblasts of the C2C12 cell line and mouse primary myoblasts derived from satellite cells—muscle stem cells playing key role in muscle regeneration. Materials with different elastic moduli within the MPa scale based on polydimethylsiloxane (PDMS) were used as cell substratum and characterized for surface roughness, wettability, and micromechanical characteristics. We found that surface properties of PDMS substrates are alter nonlinearly with the increase of the material's elastic modulus. Using this system we provide an evidence that materials with elastic modulus higher than that of physiological skeletal muscle tissue do not perturb myogenic differentiation of both types of myoblasts; thus, can be used as biomaterials for muscle tissue engineering. PDMS materials with elasticity within the range of 2.5–4 MPa may transiently limit the proliferation of myoblasts, but not the efficiency of their differentiation. Direct correlation between substratum elasticity and myogenic differentiation efficiency was not observed but the other surface properties of the PDMS materials such as nanoroughness and wettability were also diverse.

G7.12. Hristova-Panusheva, K., Keremidarska-Markova, M., Krasteva, N., Differential Effect of Novel Plant Cystatins on the Adhesive Behaviour of Normal and Cancer Breast Cells, *International Journal Bioautomation*, 2024, 28(1), pp. 59–67, ISSN 13141902, DOI 10.7546/ijba.2024.28.1.000971, **SJR 2023 0.139, Q4 12 p.**
https://www.scopus.com/record/display.uri?eid=2-s2.0-85190236753&origin=recordpage

Abstract: In the present work, we have investigated a novel recombinant cystatin dgECP1 and its mutant form, dgECP1m1, focused on their impact on the adhesive behaviour of two breast cell lines: the cancerous, MDA-MB-231, and the normal, MCF-10A. DgECP1 cystatin is intriguing with its RGD motif, responsible for cell adhesion and typical for mammalian extracellular matrix proteins but uncommon for plant cystatins. The presence of the RGD sequence suggests the potential of the dgECP1 to influence the adhesion of cancer cells and, respectively, cancer metastasis. A mutant form of the dgECP1cystatin, dgECP1m1, where RGD is replaced with HGD tripeptide, was also investigated. We found that both phytocystatins exerted differential effects on the adhesion behaviour of normal and cancer cells. In the case of dgECP1 cystatins, the effect on cancer cell adhesion also depends on the mode of administration of the cystatin to cells. When dgECP1 is pre-adsorbed on a substrate, it improves the attachment of breast cancer cells and induces cell aggregation, which is more typical for normal breast cells, and oppositely suppressed adhesion of cancer cells when added to the medium. The mutant form, dgECP1m1, inhibited cancer cell adhesion independently on the way of administration. On the other hand, both plant cystatins only slightly reduced the adhesion of normal mammary cells pointing to the higher sensitivity of cancer cells to both cystatins. These preliminary results open the possibility of considering the plant cystatin dgECP1 for anti-cancer strategies.

G7.13. Krasteva, N.; Shkodrova, M.; Keremidarska-Markova, M.; Doncheva-Stoimenova, D.; **Hristova-Panusheva, K.;** Mishonova, M.; Chichova, M. Effect of Graphene Oxide and Ammonia-modified Graphene Oxide Particles on ATPase Activity of Rat Liver Mitochondria, *International Journal Bioautomation,* 2024, 28(1), pp. 45–58, ISSN 13141902, DOI 10.7546/ijba.2024.28.1.000957, **SJR 2023 0.139, Q4 12 p**.
https://biomed.bas.bg/bioautomation/2024/vol_28.1/files/28.1_03.pdf

Abstract: Graphene and its derivatives have become promising materials for biomedical applications in the last decade. Before their widespread application, however, evaluating their toxicity and mechanisms underlying interactions with cellular components is imperative. Aims: Assessment of the effect of two graphene derivatives, pristine graphene oxide (GO) and ammonia-modified GO (GO-NH_2) particles, on the ATPase activity of rat liver mitochondria and ROS production. Methods: Liver mitochondria were isolated from male albino rats and treated with different concentrations of GO and GO-NH_2 particles (4, 10, 25, and 50 μg/ml). ATPase activity of both, intact and uncoupled by freezing/thawing mitochondria was determined by the measurement of inorganic phosphate (Pi) released from ATP. The generation of hydrogen peroxide (H_2O_2) after exposure of mitochondria to GO and GO-NH_2 particles was determined by a DCFH-D assay. Results: GO and GO-NH_2 particles applied at concentrations of 4 and 50 μg/ml did not affect the ATPase activity of intact mitochondria. In contrast, in uncoupled mitochondria, they demonstrated a stimulating effect on ATPase activity. The impact of GO-NH_2 was more substantial and concentrationdependent. ROS production was also higher in GO-NH_2-treated mitochondria. Conclusion: The present study demonstrated that GO and GO-NH_2 particles can exert a cytotoxic effect on mitochondria even after a short-time of exposure to both types of particles.

G7.14. Bela Vasileva; Natalia Krasteva; **Kamelia Hristova-Panusheva**; Penyo Ivanov; George Miloshev; Atanas Pavlov; Vasil Georgiev; Milena Georgieva, Exploring the Biosafety Potential of *Haberlea rhodopensis Friv. In Vitro* Culture Total Ethanol Extract: A Comprehensive Assessment of Genotoxicity, Mitotoxicity, and Cytotoxicity for Therapeutic Applications, *Cells*, 2024, 13(13), 1118, ISSN 20734409, DOI 10.3390/cells13131118, **IF 2024 5.1, Q1 25 p.**
https://www.mdpi.com/2073-4409/13/13/1118

Abstract: The escalating elderly population worldwide has prompted a surge of interest in longevity medicine. Its goal is to interfere with the speed of ageing by slowing it down or even reversing its accompanying effects. As a field, it is rapidly growing and spreading into different branches. One of these is the use of nutraceuticals as anti-ageing drugs. This field is gaining massive popularity nowadays, as people are shifting towards a more natural approach to life and seeking to use natural products as a source of medicine. The present article focuses on the cellular effect of *Haberlea rhodopensis* Friv. in vitro culture total ethanol extract (HRT), produced by a sustainable biotechnological approach. The extract showed a similar phytochemical profile to plant leaf extract and was rich in primary bioactive ingredients—caffeoyl phenylethanoid glycosides, myconoside, and paucifloside. This study examined the biosafety potential, cytotoxicity, genotoxicity, and mitochondrial activity of the extract using in vitro cultures. The results showed high cell survival rates and minimal cytotoxic effects on Lep3 cells, with no induction of reactive oxygen species nor genotoxicity. Additionally, the extract positively influenced mitochondrial activity, indicating potential benefits for cellular health. The results are promising and show the beneficial effect of HRT without the observation of any adverse effects, which sets the

foundation for its further testing and potential therapeutic applications.

Publications in indicator G8

Г8.1. Milena Keremidarska, **Kamelia Hristova**, Todor Hikov, Ekaterina Radeva, Dimitar Mitev, Ivailo Tsvetanov, Radina Presker,Damjana Drobne, Barbara Drašler, Sara Novak, Veno Kononenko, Kristina Eleršič, Lilyana Pramatarova, Natalia Krasteva, Development of Polymer/Nanodiamond Composite Coatings to Control Cell Adhesion, Growth, and Functions, *Advances in Planar Lipid Bilayers and Liposomes*, 2015, 21, pp. 1–26, /doi.org/10.1016/bs.adplan.2015.01.001, **book chapter 15 p.**
https://www.sciencedirect.com/science/article/abs/pii/S1554451615000022?via%3Dihub

Abstract: The identification of biomaterials that support appropriate cellular attachment, proliferation, and functions is critical for tissue engineering and cell therapy. There is a growing interest in functional organic/inorganic composites where a small amount of nanometer-sized material yields better physicochemical properties for cells to attach, grow, and differentiate. In this work, we prepared polymer/nanodiamond composite layers based on hexamethyldisiloxane and detonation-generated nanodiamond (DND) particles, in which the particles were either embedded into a polymer matrix or deposited on the preliminary formed plasma-polymerized (PP) layer. The surface properties of composites, such as roughness and wettability, as well as adhesion, growth, and functions of osteosarcoma MG-63 cells and primary rat mesenchymal stem cells were studied. We aimed to investigate the influence of the incorporation methods of DND into the polymer on the material surface properties and the cell response in order to control them by manipulating diamond-containing composite surfaces. We found differences between both composites in respect to their physicochemical properties and to the cell behavior suggesting that the method of particle incorporation into polymers should be taken in account during the development of new biomaterials for a specific application.