# Summaries

## of scientific publications
## of Assoc. Prof. Dr. Natalia Krasteva
## for participation in a competition for the academic position of "professor", published in the State Gazette, issue 109 of 21.12.2021

**B1.** M. Keremidarska-Markova, K. Hristova-Panusheva, T. Andreeva, G. Speranza, D. Wang, and N. Krasteva (2018) Cytotoxicity Evaluation of Ammonia-Modified Graphene Oxide Particles in Lung Cancer Cells and Embryonic Stem Cells. *Advances in Condensed Matter Physics*, 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-NH2) 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 μ/ml) for different times (24 and 48h). Compared with GO, GO-NH2 particles possessed smaller size, positive surface charge and higher thickness. An increased propensity to aggregation in cell cultures was also found for GO-NH2 particles. Cytotoxicity evaluation revealed that GO-NH2 particles are more toxic than pristine GO. Applied at concentrations of 10, 20 and 50 μ g/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 aremore toxic than the pristine GO which should be taken into account for future biomedical applications.

**B2.** K. Hristova-Panusheva, M. Keremidarska-Markova, T. Andreeva, G. Speranza, D. Wang, M. Georgieva, G. Miloshev and N. Krasteva (2019) Dose-dependent genotoxicity of ammonia-modified graphene oxide particles in lung cancer cells. *J Phys:Conf Ser,* 1186: 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-NH2) 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-NH2 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-NH2 NPs is important to avoide adverse biological effects.

**B3.** N. Krasteva, M. Keremidarska-Markova, K. Hristova-Panusheva, T. Andreeva, G. Speranza, D. Wang, M. Draganova-Filipova, G. Miloshev and M. Georgieva (2019) Aminated Graphene Oxide as a Potential New Therapy for Colorectal Cancer, *Oxidative Med Cell Longev*, 2019;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-NH2) 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.

**B4.** M. Georgieva, B. Vasileva, G. Speranza, D. Wang, K. Stoyanov, M. Draganova-Filipova, P. Zagorchev, V. Sarafian, G. Miloshev and N. Krasteva (2020) Amination of graphene oxide leads to increased cytotoxicity in hepatocellular carcinoma cells. *Int J Mol Sci* , 21(7), 2427.

**Abstract:** Clinically, there is an urgent need to identify new therapeutic strategies for selectively treating cancer cells. One of the directions in this research is the development of biocompatible therapeutics that selectively target cancer cells. Here, we show that novel aminated graphene oxide (haGO-NH2) nanoparticles demonstrate increased toxicity towards human hepatocellular cancer cells compared to pristine graphene oxide(GO). The applied novel strategy for amination leads to a decrease in the size of haGO-NH2 and their zeta potential, thus, assuring easier penetration through the cell membrane. After characterization of the biological activities of pristine and aminated GO, we have demonstrated strong cytotoxicity of haGO-NH2 toward hepatic cancer cells—HepG2 cell line, in a dose-dependent manner. We have presented evidence that the cytotoxic e_ects of haGO-NH2 on hepatic cancer cells were due to cell membrane damage, mitochondrial dysfunction and increased reactive oxygen species (ROS) production.

Intrinsically, our current study provides new rationale for exploiting aminated graphene oxide as an anticancer therapeutic.

**B5.** N. Krasteva, D. Staneva, B. Vasileva, G. Miloshev and M. Georgieva (2021) Bioactivity of pegylated graphene oxide nanoparticles combined with near-infrared laser irradiation studied in colorectal carcinoma cells. *Nanomaterials*, 11(11): 3061.

**Abstract:** Central focus in modern anticancer nanosystems is given to certain types of nanomaterials such as graphene oxide (GO). Its functionalization with polyethylene glycol (PEG) demonstrates high delivery efficiency and controllable release of proteins, bioimaging agents, chemotherapeutics and anticancer drugs. GO–PEG has a good biological safety profile, exhibits high NIR absorbance and capacity in photothermal treatment. To investigate the bioactivity of PEGylated GO NPs in combination with NIR irradiation on colorectal cancer cells we conducted experiments that aim to reveal the molecular mechanisms of action of this nanocarrier, combined with near-infrared light (NIR) on the high invasive Colon26 and the low invasive HT29 colon cancer cell lines. During reaching cancer cells the phototoxicity of GO–PEG is modulated by NIR laser irradiation. We observed that PEGylation of GO nanoparticles has well-pronounced biocompatibility toward colorectal carcinoma cells, besides their different malignant potential and treatment times. This biocompatibility is potentiated when GO–PEG treatment is combined with NIR irradiation, especially for cells cultured and treated for 24 h. The tested bioactivity of GO–PEG in combination with NIR irradiation induced little to no damages in DNA and did not influence the mitochondrial activity. Our findings demonstrate the potential of GO–PEG-based photoactivity as a nanosystem for colorectal cancer treatment.

**B6.** Z. Gospodinova, T. Kamenska, Gencheva G, M. Georgieva, N. Krasteva. PEGylation of graphene oxide nanosheets modulate cancer cell motility and proliferative ability, *Journal of Physics: Conference Series*, 2021, 1762(1), 012001, SJR:0.227.

**Abstract:** Recently, graphene oxide (GO) has been increasingly investigated for its biomedical and biological applications, including cancer research. The interest is set on GO chemical modifications and their implications in the development of therapeutic approaches for various diseases. Recent data have demonstrated that PEGylation of nanoparticles (NPs) improves NPs solubility and stability in physiological solutions and alters their reactivity toward cancer cells. In this work, we have evaluated the effect of PEGylated GO nanosheets on the migratory and proliferation ability of A375 melanoma cells, used as a cancer cell model and have compared it to normal kidney MDCK cells. Both types of GOs, pristine and PEGylated, demonstrated an inhibitory effect on the cancer cells proliferation and mobility while on normal MDCK cells the effect of GO was significantly weaker at 48 hours of exposure suggesting that cancer A375 cells were more sensitive to GO and GO-PEG treatment. In general, PEGylation mitigates the inhibitory effect of GO on the growth and migratory ability of melanoma cells. Our results prove that the effects of both GOs NPs on cancer cells proliferation and mobility are dose-, NPs- and cell-type-dependent, hence providing a rationale for future design and use of graphene-based nanomaterials for cancer research.

**B7.** M. Georgieva; Z. Gospodinova; M. Keremidarska-Markova; T. Kamenska; G. Gencheva and N. Krasteva (2021) PEGylated nanographene oxide in combination with near-Infrared laser irradiation as a smart nanocarrier in colon cancer targeted therapy. *Pharmaceutics*, 13(3): 424

**Abstract:** Anti-cancer therapies that integrate smart nanomaterials are the focus of cancer research in recent years. Here, we present our results with PEGylated nanographene oxide particles (nGO-PEG) and have studied their combined effect with near-infrared (NIR) irradiation on low and high invasive colorectal carcinoma cells. The aim is to develop nGO-PEG as a smart nanocarrier for colon cancertargeted therapy. For this purpose, nGO-PEG nanoparticles' size, zeta potential, surface morphology, dispersion stability, aggregation, and sterility were determined and compared with pristine nGO nanoparticles (NPs). Our results show that PEGylation increased the particle sizes from 256.7 nm (pristine nGO) to 324.6 nm (nGO-PEG), the zeta potential from -32.9 to -21.6 mV, and wrinkled the surface of the nanosheets. Furthermore, nGO-PEG exhibited higher absorbance in the NIR region, as compared to unmodified nGO. PEGylated nGO demonstrated enhanced stability in aqueous solution, improved dispensability in the culture medium, containing 10% fetal bovine serum (FBS) and amended biocompatibility. A strong synergic effect of nGO-PEG activated with NIR irradiation for 5 min (1.5 W/cm2 laser) was observed on cell growth inhibition of low invasive colon cancer cells (HT29) and their wound closure ability while the effect of NIR on cellular morphology was relatively weak. Our results show that PEGylation of nGO combined with NIR irradiation holds the potential for a biocompatible smart nanocarrier in colon cancer cells with enhanced physicochemical properties and higher biological compatibility. For that reason, further optimization of the irradiation process and detailed screening of nGO-PEG in combination with NIR and chemotherapeutics on the fate of the colon cancer cells is a prerequisite for highly efficient combined nanothermal and photothermal therapy for colon cancer.

**Γ1.** T. Kamenska, M. Abrashev, M. Georgieva and N. Krasteva (2021) Impact of Polyethylene Glycol Functionalization of Graphene Oxide on Anticoagulation and Haemolytic Properties of Human Blood. *Materials*, *14*(17), 4853.

**Abstract:** Graphene oxide (GO) is one of the most explored nanomaterials in recent years. It has numerous biomedical applications as a nanomaterial including drug and gene delivery, contrast imaging, cancer treatment, etc. Since most of these applications need intravenous administration of graphene oxide and derivatives, the evaluation of their haemocompatibility is an essential preliminarystep for any of the developed GO applications. Plentiful data show that functionalization of graphene oxide nanoparticles with polyethylene glycol (PEG) increases biocompatibility, thus allowing PEGylated GO to elicit less dramatic blood cell responses than their pristine counterparts. Therefore, in this work, we PEGylated graphene oxide nanoparticles and evaluated the effects of their PEGylation on the structure and function of human blood components, especially on the morphology and the haemolytic potential of red blood cells (RBCs). Further, we studied the effect of PEGylation on some blood coagulation factors, including plasma fibrinogen as well as on the activated partial thromboplastin (aPTT), prothrombin time (PT) and platelet aggregation. Our findings provide important information on the mechanisms through which PEGylation increases GO compatibility with human blood cells. These data are crucial for the molecular design and biomedical applications of PEGylated graphene oxide nanomaterials in the future.

**Γ2.** G. Xiao, H. Chen, N. Krasteva, Q. Liu and D. Wang (2018) Identification of interneurons required for the aversive response of Caenorhabditis elegans to graphene oxide. *Journal of Nanobiotechnology*, 16(1):45.

**Abstract:** Background: So far, how the animals evade the environmental nanomaterials is still largely unclear. In this study, we employed in vivo assay system of Caenorhabditis elegans to investigate the aversive behavior of nematodes to graphene oxide (GO) and the underlying neuronal basis. Results: In this assay model, we detected the significant aversive behavior of nematodes to GO at concentrations more than 50 mg/L. Loss-of-function mutation of nlg-1 encoding a neuroligin with the function in connecting preand post-synaptic neurons suppressed the aversive behavior of nematodes to GO. Moreover, based on the neuronspecific activity assay, we found that the NLG-1 activity in AIY or AIB interneurons was required for the regulation of aversive behavior to GO. The neuron-specific activities of NLG-1 in AIY or AIB interneurons were also required for the regulation of GO toxicity. Conclusions: Using nlg-1 mutant as a genetic tool, we identified the AIY and AIB interneurons required for the regulation of aversive behavior to GO. Our results provide an important neuronal basis for the aversive response of animals to environmental nanomaterials.

**Γ3.** M. Ren, L. Zhao, X. Ding, N. Krasteva, Q. Rui and D. Wang (2018) Developmental basis for intestinal barrier against the toxicity of graphene oxide. *Particle and Fibre Toxicology* 15(1):26.

**Abstract:** Background: Intestinal barrier is crucial for animals against translocation of engineered nanomaterials (ENMs) into secondary targeted organs. However, the molecular mechanisms for the role of intestinal barrier against ENMs toxicity are still largely unclear. The intestine of Caenorhabditis elegans is a powerful in vivo experimental system for the study on intestinal function. In this study, we investigated the molecular basis for intestinal barrier against toxicity and translocation of graphene oxide (GO) using C. elegans as a model animal. Results: Based on the genetic screen of genes required for the control of intestinal development at different aspects using intestine-specific RNA interference (RNAi) technique, we identified four genes (erm-1, pkc-3, hmp-2 and act-5) required for the function of intestinal barrier against GO toxicity. Under normal conditions, mutation of any of these genes altered the intestinal permeability. With the focus on PKC-3, an atypical protein kinase C, we identified an intestinal signaling cascade of PKC-3-SEC-8-WTS-1, which implies that PKC-3 might regulate intestinal permeability and GO toxicity by affecting the function of SEC-8-mediated exocyst complex and the role of WTS-1 in maintaining integrity of apical intestinal membrane. ISP-1 and SOD-3, two proteins required for the control of oxidative stress, were also identified as downstream targets for PKC-3, and functioned in parallel with WTS-1 in the regulation of GO toxicity. Conclusions: Using C. elegans as an in vivo assay system, we found that several developmental genes required for the control of intestinal development regulated both the intestinal permeability and the GO toxicity. With the focus on PKC-3, we raised two intestinal signaling cascades, PKC-3-SEC-8-WTS-1 and PKC-3-ISP-1/SOD-3. Our results will strengthen our understanding the molecular basis for developmental machinery of intestinal barrier against GO toxicity and translocation in animals.

**Γ4.** L. Zhao, J. Kong, N. Krasteva and D. Wang (2018) Deficit in the epidermal barrier induces toxicity and translocation of PEG modified graphene oxide in nematodes., *Toxicology Research*, 7(6):1061-1070.

**Abstract:** The developmental basis for the epidermal barrier against the translocation of nanomaterials is still largely unclear in organisms. We here investigated the effect of deficits in

the epidermal barrier on the translocation and toxicity of PEG modified graphene oxide (GO-PEG) in Caenorhabditis elegans. In wild-type or NR222 nematodes, GO-PEG exposure did not cause toxicity and affect the expression of epidermaldevelopment related genes. However, GO-PEG exposure resulted in toxicity in mlt-7(RNAi) nematodes with deficit in the function of epidermal barrier. Epidermal RNAi knockdown of mlt-7 allowed GO-PEG accumulation and translocation into targeted organs through the epidermal barrier. Epidermal-development related proteins of BLI-1 and IFB-1 were identified as targets for MLT-7 in the regulation of GO-PEG toxicity and accounted for MLT-7 function in maintaining the epidermal barrier. AAK-2, a catalytic α subunit of AMP-activated protein kinase, was identified as another target for MLT-7 in the regulation of GO-PEG toxicity. AAK-2 functioned synergistically with BLI-1 or IFB-1 in the regulation of GO-PEG toxicity. Our data provide the molecular basis for the role of epidermal barrier against the toxicity and translocation of nanomaterials in organisms.

**Γ5.** L. Zhao, S. Dong, Y. Zhao, H. Shao, N. Krasteva, Q. Wu and D. Wang (2019), Dysregulation of let-7 by PEG modified graphene oxide in nematodes with deficit in epidermal barrier. *Ecotoxicology and Environmental Safety*, 169, 1-7. DOI:10.1016/j.ecoenv.2018.10.106.

**Abstract**: In nematode Caenorhabditis elegans, epidermal RNA interference (RNAi) knockdown of bli-1 encoding a cuticular collagen caused the toxicity induction of GO-PEG (PEG surface modified graphene oxide). In this study, we further found that epidermal RNAi knockdown of bli-1 increased expression of a microRNA let-7, and let-7 mutation suppressed the susceptibility of bli-1(RNAi) nematodes to GO-PEG toxicity. let-7 regulated the toxicity induction of GO-PEG by suppressing expression and function of its direct targets (HBL-1 and LIN-41). Like the nematodes with epidermal RNAi knockdown of bli-1, epidermal RNAi knockdown of hbl-1 or lin-41 also induced functional abnormality in epidermal barrier. Therefore, a signaling cascade of BLI-1-let-7-HBL-1/LIN-41 was raised to be involved in GO-PEG toxicity induction. Our data imply the dysregulation of let-7-mediated molecular machinery for developmental timing control by GO-PEG in nematodes with deficit in epidermal barrier causedby bli-1(RNAi).

**Γ6.** H. Shao, Z. Han, N. Krasteva and D. Wang (2019) Identification of signaling cascade in the insulin signaling pathway in response to nanopolystyrene particles, *Nanotoxicology*, 13(2):174-188.

**Abstract:** The molecular response of animals to nanoplastic particles is still largely unclear. In this study, we employed a modified prolonged exposure system to investigate the molecular response of Caenorhabditis elegans to nanopolystyrene particles. Exposure to nanopolystyrene particles (1 μg/L) significantly decreased expressions of daf-2 encoding an insulin receptor, age-1 encoding a PI3K, and akt-1 encoding an Akt/PKB, and increased expression of daf-16 encoding a FOXO transcriptional factor in insulin signaling pathway. Among these genes, mutation of daf-2, age-1, or akt-1 induced a resistance to toxicity of nanopolystyrene particles, whereas mutation of daf-16 induced a susceptibility to the toxicity of nanopolystyrene particles. RNAi knockdown of daf-16 could further suppress the resistance of daf-2, age-1, or akt-1 mutant to the toxicity of nanopolystyrene particles. The insulin signaling pathway acted in intestinal cells to regulate the toxicity of nanopolystyrene particles. Moreover, sod-3 encoding a manganese superoxide dismutase, mtl-1 encoding a metallothionein, and gpd-2 encoding a glyceraldehyde-3-phosphate dehydrogenase were identified as downstream targeted genes for daf-16 in the regulation of toxicity of nanopolystyrene particles. Therefore, a signaling cascade

of DAF-2-AGE-1-AKT-1-DAF- 16-SOD-3/MTL-1/GPD-2 was identified in response to nanopolystyrene particles in nematodes. Additionally, this signaling cascade in the insulin signaling pathway may mediate a protective response for nematodes against the adverse effects from nanopolystyrene particles.

**Г7.** M. Keremidarska-Markova, E. Radeva, D. Mitev, K. Hristova-Panusheva, B. Paull, P. Nesterenko, J. Šepitka, I. Junkar, A. Iglič and N. Krasteva (2018) Increased elastic modulus of plasma polymer coatings reinforced with detonation nanodiamond particles improves osteogenic differentiation of mesenchymal stem cells. *Turkish Journal of Biology,* 42(2): 195.

**Резюме:** В настоящото изследване ние демонстрирахме, че композитните PPHMDS/DND покрития с еластични модули, близки до тези на костната тъкан на възрастни индивиди (0,2–2,8 GPa) стимулират растежа и остеогенната диференциация на мезенхимни стволови клетки, получени от човешки мастни клетки (hADMSCs). Композитните покрития бяха получени по метода на плазмената полимеризация (PP), където детонационни нанодиамантени (DND) частици в различни количества (0,1, 0,5 и 1 mg/mL) бяха добавени към хексаметилдисилоксан (HMDS) преди плазмено отлагане. Този метод позволява промяна само в модула на еластичност (Er′), с увеличаване на концентрацията на частиците, докато другите повърхностни свойства, включително повърхностно омокряне и топография, не се променят. Отговорът на hAD-MSCs към нарастващата твърдост показва ефект върху адхезията и остеогенната диференциация, но не и върху клетъчната пролиферация. Минерализацията на екстрацелуларния матрикс и разпределението на клетките бяха най-високи върху PPHMDS/DND покрития с най-висок модул на еластичност (2,826 GPa), докато разликите в пролиферацията между отделните покрития бяха незначителни. Като цяло, PPHMDS/DND покритията осигуряват по-добри условия за растеж и остеогенна диференциация на hAD-MSCs в сравнение със покривните стъкла, използвани като положителни контроли, потвърждавайки тяхната възможност за приложения за остео-интеграция. Освен това, нашите резултати подкрепят хипотезата, че биоматериали с еластичност, близка до тази на нативната тъкан, могат да подобрят диференциацията на мезенхимните стволови клетки.

**Г8.** B. Świerczek-Lasek, M. Keremidarska-Markova, K. Hristova-Panusheva, T. Vladkova, M. A. Ciemerych, K. Archacka and N. Krasteva (2019) Polydimethylsiloxane materials with supraphysiological elasticity enable differentiation of myogenic cells. *J Biomed Mater Res A,* 107:2619-28.

**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.

**Г9.** T. Hikov, N. Krasteva, K. Hristova-Panusheva**,** N. Ivanov and P. Petrov (2019) Study on the biocompatibility of $TiN/TiO_2$ bilayer coatings deposited by DC magnetron sputtering on stainless steel, *AIP Conference Proceedings,* 2075, 160022.

**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.

**Г10.** A. A. Haroun, Z. Gospodinova, N. Krasteva (2021) Amino Acid Functionalization of Multi-Walled Carbon Nanotubes for Enhanced Apatite Formation and Biocompatibility, *Nano Biomedicine and Engineering*.13 (4):380-393, SJR-0.252.

**Abstract:** The limitation in bone tissue engineering is the lack of available natural or synthetic biomaterials to replace bone tissue under need. Carbon nanotubes have great potential as bone tissue scaffolds because of their remarkable mechanical and electrical properties combined with high aspect ratio. In this work, we demonstrated for the first time a novel approach based on the sol-gel technique for functionalization of multi-walled carbon nanotubes (MWCNTs) with two amino acids: L-arginine, L(+) Arg and L-aspargine, L(+) Asp. We have examined the effect of both functionalities on physico-chemical properties of MWCNTs, cytotoxicity in osteosarcoma MG63 and normal fibroblastic BJ cells and the ability to induce nucleation and growth of hydroxyapatite (HA) crystals *in vitro* under physiological concentrations of Ca2+ and PO4+ (SBF). The scaffolds were characterized using Fourier transform infrared spectroscopy (FTIR-ATR), dynamic light scattering technique (DLS), X-ray diffraction (XRD), thermogravimetric analysis (TGA) and scanning electron microscopy (SEM). The results showed that both functionalized MWCNTs have a particle size of 269 and 411 nm, a zeta potential of –12.8 and –8.8 mV, respectively, high colloidal stability, enhanced biocompatibility, and enhanced formation of an apatite layer on the scaffolds surface in comparison to ox-MWCNTs. Altogether, the results confirmed the important role of the amino acids L(+) Arg and L(+) Asp in ox-MWCNTs-based composites for bone tissue engineering applications.

**Г11.** D. Gugutkov, F. Awaja, K. Belemezova, M. Keremidarska, N. Krasteva, S. Kuyrkchiev, G. GallegoFerrer, S. Seker, A.E. Elcin, Y.M. Elcin and G. Altankov (2017) Osteogenic differentiation of mesenchymal stem cells using hybrid nanofibers with different configurations and dimensionality. *J Biomed Mater Res Part A*, 1, 552-4965.

**Abstract:** Novel, hybrid fibrinogen/polylactic acid (FBG/PLA) nanofibers with different configuration (random vs aligned) and dimensionality (2-D vs 3-D environment) were used to control the overall behavior and the osteogenic differentiation of human adipose-derived mesenchymal stem cells (ADMSCs). Aligned nanofibers in both the 2-D and 3-D configurations are proved to be favored for osteodifferentiation. Morphologically, we found that on randomly configured nanofibers, the cells developed a stellate-like morphology with multiple projections; however, time-lapse analysis showed significantly diminished cell movements. Conversely, an elongated cell shape with advanced cell spreading and extended actin cytoskeleton accompanied with significantly increased cell mobility were observed when cells attached on aligned nanofibers. Moreover, a clear tendency for higher alkaline phosphatase activity was also found on aligned fibers when ADMSCs were switched to osteogenic induction medium. The strongest accumulation of Alizarin red (AR) and von Kossa stain at 21 days of culture in osteogenic medium were found on 3-D aligned constructs while the rest showed lower and rather undistinguishable activity. Quantitative reverse transcription-polymerase chain reaction analysis for Osteopontin (OSP) and RUNX 2 generally confirmed this trend showing favorable expression of osteogenic genes activity in 3-D environment particularly in aligned configuration.

**Г12.** B. Vasileva, D. Staneva, N. Krasteva, G. Miloshev and M. Georgieva (2021) Changes in chromatin organization eradicate cellular stress resilience to irradiation with UVA/B light and induce premature ageing, *Cells*, 10(7): 1755.

**Abstract:** Complex interactions among DNA and nuclear proteins maintain genome organization and stability. The nuclear proteins, particularly the histones, organize, compact, and preserve the stability of DNA, but also allow its dynamic reorganization whenever the nuclear processes require access to it. Five histone classes exist and they are evolutionarily conserved among eukaryotes. The linker histones are the fifth class and over time, their role in chromatin has been neglected. Linker histones interact with DNA and the other histones and thus sustain genome stability and nuclear organization. Saccharomyces cerevisiae is a brilliant model for studying linker histones as the gene for it is a single-copy and is non-essential. We, therefore, created a linker histone-free yeast strain using a knockout of the relevant gene and traced the way cells age chronologically. Here we present our results demonstrating that the altered chromatin dynamics during the chronological lifespan of the yeast cells with a mutation in ARP4 (the actin-related protein 4) and without the gene HHO1 for the linker histone leads to strong alterations in the gene expression profiles of a subset of genes involved in DNA repair and autophagy. The obtained results further prove that the yeast mutants have reduced survival upon UVA/B irradiation possibly due to the accelerated decompaction of chromatin and impaired proliferation. Our hypothesis posits that the higher-order chromatin structure and the interactions among chromatin proteins are crucial for the maintenance of chromatin organization during chronological aging under optimal and UVA-B stress conditions.

**Г13.** M. Keremidarska, K. Hristova, T. Hikov, E. Radeva, D. Mitev, I. Tsvetanov, R. Presker, D. Drobne, B. Drašler, S. Novak, V. Kononenko, K. Eleršič, L. Pramatarova and N. Krasteva.

(2015) Development of Polymer/Nanodiamond Composite Coatings to Control Cell Adhesion, Growth and Functions In: *Advances in Planar Lipid Bilayers and Liposomes*. Elsevier. Edds: *A. Iglič, Ch.V. Kulkarni, M. Rappol,.* 21, 1-26 -book chapter.

**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.