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ABSTRACTS

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Bio Applications/Nanomedicine

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***** INVITED LECTURE *****

Nanomedicine: Present Accomplishments and Prospects (Quantum Dots) ***C1***

In modern-day medicine, nanotechnology and nanoparticles are used for the diagnosis, prevention, and treatment of disease and to gain a better understanding of underlying human disease mechanisms. There are many different types of nanomaterials are used in medicine, each of which can be modified in a infinite number of ways. It would therefore be overwhelming to attempt to create a comprehensive description of all the materials that could potentially be used in nanomedicine. It is worth mentioning many reviews and articles about quantum dots (QD) as very attractive and prospects materials for research of biomedical tasks. In this review, we will try to generelize and discuss the present accomplishments and prospects in the utility and applications of QD in nanomedicine. We will summarize general and particular requirements for use QD in medicine (surface passivation, adsorption, functionalization, bioconjugation, uptake or incorporation of QD by living cells etc.), using of QDots as imaging agent of ions, single molecules, cellular structures and receptors, individual cells and tissue along with targeting, tracking, distribution etc., properties of QDs as multimodality imaging probes, multifunctionality QD as good candidates for theranostic platforms (the combination of a QD imaging agent with a therapeutic agent, the use of QD in studying drug release, QDs as sensitizers for photodynamic therapy, use of luminescent QD to initiate biological functions etc.), using of QD as part of sensors without function of imaging agent. In addition, main concerns about the potential toxicity of QDs are also introduced.

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The Optimization of Microchip Digital Polymerase Chain Reaction with Microwell Sample Dispersion ***C2***

The digital polymerase chain reaction (dPCR) rising in development and popularity. dPCR is a suitable solution for the analysis of rare samples or samples available only in small quantities. The determination of absolute copy number of target DNA without the use of external standards together with the possibility of statistical analysis due to the binary nature of the data are the main advantages of dPCR. Chip dPCR compared with droplet dPCR is less widely used and developed. The precision and dynamic range of dPCR is dependent on the number of reaction wells. The increment of the number of reaction wells on chip, leads to the issue of appropriate method of sample loading. Our designed and fabricated silicon chips with 26 448 microwells with the volume of one microwell 59 pL was tested for suitable method of sampling for our experimental dPCR technology. During incorrect sample loading on chip surface, it arises to the amplification defects e.g. evaporation of sample, cluster formation, uneven fluorescence intensity or unfilled wells which led to incorrect amplification and subsequent quantification. We verified couple of sampling methods to improve the loading efficiency and precision of quantification. Our optimized method of sample loading for our dPCR technology was developed. The dPCR chip was filled with the 4 µL of PCR master mix, covered with 10 µL of mineral oil and the modified covered glass.

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Iron Oxide Nanoparticles Cause Inflammatory Response in Murine Renal Podocytes Depending on the Type of Coating ***C3***

Theranostics is a new field of medicine, which combines specific diagnostic and therapeutic methods. It is based on the use of nanoparticles, e.g. iron oxide nanoparticles, which can be divided based on size, shape, type of core, shell, etc [1].

Nanoparticles (NPs) in the blood might reach and accumulate in the kidneys potentially leading to disruption of whole body homeostasis and therefore it is important to know their nephrotoxicity [2]. We investigated the effect of two types of iron oxide nanoparticles with the same core (7.6 nm) coated with either polyethylene glycol (PEG) or bovine serum albumin (BSA) on specific renal glomerular cells - podocytes. Both types of nanoparticles triggered different effects on cells, although they were of the same material and the same size of the core. PEG-coated NPs were approx. five times more toxic than BSA-coated NPs. Inflammation has also been observed. mRNA expression of proinflammatory factors was detected 5-hour after treatment of PEG coated iron oxide nanoparticles, but not after treatment with BSA-coated NPs. The opposite effect was observed after a 24-hour exposure when only BSA-coated nanoparticles increased the expression level of pro-inflammatory factors. We observed the different impact of both types of NPs on podocytes also in other experiments. Our results show that the coating of nanoparticles is as important for the resulting functional nanoparticle as other variables, if not more. A full understanding of nano:bio interactions in a living system, especially in excretion organs like kidneys will be needed for successful implementation of nanoparticles in the treatment of diseases. ACKNOWLEDGEMENTS: This work was supported by the Slovak Research and Development Agency under the contract No. APVV-16-0579 This study was performed during the implementation of the project Buildingup Centre for advanced materials application of the Slovak Academy of Sciences, ITMS project code 313021T081 supported by Research & Innovation Operational Programme funded by the ERDF. REFERENCES: [1] Xie J, Lee S, Chen X. Nanoparticle-based theranostic agents. Adv Drug Deliv Rev. 2010;62(11):1064-1079. doi:10.1016/j.addr.2010.07.009.; [2] Rana SVS (2021) Recent Advances on Renal Toxicity of Engineered Nanoparticles-A Review. J Toxicol Risk Assess 7:036. doi.org/10.23937/2572-4061.1510036.

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Mechanochemical Preparation, Characterization and Biological Activity of Stable CuS Nanosuspension Capped by Bovine Serum Albumin ***C4***

The biocompatible nanosuspension of CuS nanoparticles using bovine serum albumin (BSA) was prepared using a two-stage mechanochemical approach. CuS nanoparticles were firstly synthetized by a planetary ball milling (according to [1]) and then a wet stirred media milling in a solution of BSA was introduced to yield the CuS-BSA. The nanosuspension was stable for more than 9 months, as confirmed by photon cross-correlation spectroscopy and zeta potential measurements. The obtained nanosuspension was characterized by UV-Vis and PL spectroscopy.The fluorescent properties of the nanocrystals were confirmed by PL spectroscopy, which showed that tryptophan could be closer to the binding site than the tyrosine residue. Scanning and transmission electron microscopy confirmed the nancocrystalline character of CuS and the presence of BSA. The biological activity was determined by in vitro tests on selected cancer and healthy human cell lines. The results have shown that CuS-BSA nanosuspension inhibits the metabolic activity of the cells as well as decreases their viability upon photothermal ablation. ACKNOWLEDGEMENTS: This work was supported by the Slovak Agency for Research and Development on the basis of contract no. APVV-18-0357 and by the Scientific Grant Agency of the Ministry of Education, Science, Research and Sport (project no. 2/0044/18).

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Adsorption of Bovine Serum Albumin and Amino Acid Residues on ZnO Single-Crystal Facetes: Simulations and Microscopy ***C5***

ZnO is becoming one of the most important semiconductors in biological applications because of its specific properties such as low cost, large direct bandgap (3.37 eV), and great biocompatibility. Interactions between ZnO and different proteins

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In Vivo Biodistribution of Gold Nanospheres in Long-Term Scale ***PC24***

One of the biggest concerns of nanomedicine is the long-term fate of nanoparticles in the body. Each treatment substance should be safely eliminated from the organism after fulfilling their therapeutic purpose. The results from studies focused on nanoparticle-based treatment agents indicate their accumulation in some organs short after application [1][2], but little is known about longer time periods. Gold nanoparticles are wide studied as a promising tool for e.g. drug delivery, imaging or photothermal therapy, therefore it is important to investigate their long-term effect on the organism. In our study, we applied the sphere shaped gold nanoparticles (AuNPs) with 10 nm in diameter and coated with bovine serum albumin (BSA) to C57BL/6 mice. After intravenous administration, mice were observed and regularly weighted for 120 days. At the end of the experiment, organs - liver and spleen were chirurgical extracted and analysed. The health of mice was not affected by the AuNPs application for duration of the experiment, despite we were able to detect AuNPs in the liver and spleen tissue by atomic absorption spectrometry even after 4 months from AuNPs application. Interestingly, both organs were slightly heavier in comparison to control group and whether this could be a direct effect of accumulated AuNPs is the object of further investigation. Thus our results indicate, that AuNPs accumulate in sites of excretion - liver (the main excretory organ for particles above the renal filtration limit) and spleen (splenic clearance) and persist there at least 4 months. ACKNOWLEDGEMENTS: This work was supported by the Slovak Research and Development Agency under the contract No. APVV-16-0579 This study was performed during the implementation of the project Buildingup Centre for advanced materials application of the Slovak Academy of Sciences, ITMS project code 313021T081 supported by Research & Innovation Operational Programme funded by the ERDF. REFERENCES: [1] Bailly, A. L., Correard, F., Popov, A., Tselikov, G., Chaspoul, F., Appay, R., ... & Esteve, M. A. (2019). In vivo evaluation of safety, biodistribution and pharmacokinetics of laser-synthesized gold nanoparticles. Scientific reports, 9(1), 1-12.; [2] Brown, A. L., Kai, M. P., DuRoss, A. N., Sahay, G., & Sun, C. (2018). Biodistribution and toxicity of micellar platinum nanoparticles in mice via intravenous administration. Nanomaterials, 8(6), 410.

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Influence of PEG and PLL Linkers on Cytocompatibility of Few-Layer Black Phosphorus and Peptide Bioconjugates: Novel FLBP Nanoplatforms as Promising Agents in Cancer Therapy ***PC25***

A novel 2D nanomaterial - few-layer black phosphorus (FLBP) - was modified by peptide bioconjugates towards enhancing its therapeutic efficiency by reducing its side effects and improving its selective binding ability towards a biological target. Two various strategies of BP modification using poly-L-lysine (PLL) and NH2-PEG-OH conjugates with functionalised peptides were conducted. The application of covalent immobilization protocol to coupling PLL linker with peptide allow to stronger bond formation and more stable hybrid than physisorption. The biocompatible FLBP-PLL-peptides conjugates are synthesized according to proposed non-covalent strategy. The attachment of cationic linker with peptide to surface of black phosphorus is stabilized by electrostatic and hydrophobic interaction. The proposed non-covalent functionalization of BP surface by poly-L-lysine improved nanomaterial stability. Application of RP-HPLC analysis for preliminary screening of covalent conjugation of linker and peptide proved to be efficient and practical method to be used in further investigations. PEG was chosen as a linker for two reasons, firstly it protects FLBP structure from outer water, oxygen and non-specific interior interactions and increase stability of FLBP in physiological conditions. Furthermore, the use of non-ionic and hydrophilic linker can overcome problem of nonspecific adsorption upon the surface, e.g., by proteins from blood serum or a bioanalyte sample. Secondly, a stable immobilization of peptides on PEG linker prolonged circulation in the blood and prevent drug release during its transport which may improve pharmaceutical properties of peptides. In conclusion, we have successfully fabricated nanoparticles of BP-PEG-peptide and BP-PLL-peptide conjugations. Two types of linkers PEG and PLL were compared as a spacer molecule due to their different polymer mass, structure and biological activity. The effect of these parameters was demonstrated in the in vitro experiments.

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Antimicrobial Properties of Polymeric Nanofibrous Membranes Containing Ferrous Sulphate ***PC43***

A significant attention has been paid to polymer nanofibers, due to their beneficial properties such as a high specific surface area and high porosity. They can be used in various applications including separation processes, wound healing, tissue engineering etc. Besides others, the nanofiber-based materials have great ability to aerosol filtration and trapping microorganisms that has become extremely relevant duo to the COVID-19 occurrence. Nanofibrous membranes doped with an active antibacterial component directly in the mass of nanofibers are a next step towards products that actively eliminate microorganisms and thus protect human health. In our study, nanofibers of polymers doped with ferrous sulphate (FeSO4) or quaternary ammonium salt (QAS) were prepared by electrospinning. Three types of polymers (polyvinylidenfluoride/PVDF, polylactic acid/PLA and polyurethane/PU) with good electrospinning processability and good mechanical properties of nanofibers were chosen. The prepared nanofibrous membranes were characterized in terms of materials by FTIR, the morphology of the fibers was assessed by SEM, and the pore size was determined by porometry. The leaching test showed a firm anchoring of the additive in the nanofiber structure. Antimicrobial activity was monitored after 0 h, 0.5 h, 4 h and 24 h using Staphylococcus aureus (CCM 4516) and Klebsiella pneumoniae (CCM 4415) strains. The membrane prepared from PU doped with FeSO4 showed the best antibacterial efficiency. The porosity and morphology of the nanofibrous membrane effectively contribute to the trapping of microorganisms. This system was also evaluated as the most suitable from the electrospinning process effectivity point of view.

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Inflammatory Response of Murine Renal Mesangial Cells Depends on Magnetite Nanoparticle Coating ***PC44***

Despite benefits nanotherapeutics offer to healthcare strategies, there are still concerns about their biological safety. Kidney is responsible for elimination of toxic wastes from the blood, but nanoparticles independently of filtration can accumulate here, mostly in the glomerular mesangium. Magnetic nanoparticles (MNPs) are promising candidates for modern biomedicine. As prolonged residency of nanoparticles has been associated with the nephrotoxicity, we investigated the impact of MNPs on renal mesangial cells controlling glomerular hemodynamics and filtration process. Primary murine glomerular mesangial cells were exposed to MNPs with two different coatings - polyethylene glycol (PEG) or bovine serum albumin (BSA). PEGylated MNPs activated stronger inflammatory response compared to BSA coated MNPs. Strong induction of iNOS by PEG MNPs after 24 hours of exposure was in contrast to low-to-no induction of inflammatory factors (TNFa, IL-6, Mip2) induced by BSA MNPs. This was confirmed on mRNA as well as protein levels. Both types of MNPs activated slight actin fiber remodeling associated with weak reduction in cell adhesion properties. Mesangial cells also displayed certain signs of damage after MNPs internalization, concerning mostly endoplasmic reticulum and mitochondria. Taking together, the results indicate a crucial role of coating in MNPs-induced mesangial cell toxicity. ACKNOWLEDGMENTS: This work was supported by the Slovak Research and Development Agency under the contract No. APVV-16-0579 This study was performed during the implementation of the project Buildingup Centre for advanced materials application of the Slovak Academy of Sciences, ITMS project code 313021T081 supported by Research & Innovation Operational Programme funded by the ERDF.

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Self-Activated Antibacterial MOFs-Based Coating on Medically Relevant Polymers ***PC45***

Modern medicine is now unimaginable without the use of various assistive materials and devices such as catheters, implants, and others, which come into contact with body fluids and tissues. In this context, the potential danger of bacterial colonization on the device surfaces and the subsequent formation of biofilms can cause serious infections and diseases. In an effort to prevent bacterial colonization, the smart self-activating antibacterial coating on a medically relevant surface