SYMPOSIUM ABSTRACTS
Arh Hig Rada Toksikol 2024;75:A2-A15

Abstracts

Synergy at the chemistry-nanotechnology interface

26 May 2023
Institute for Medical Research and Occupational Health, Zagreb, Croatia

On May 26, 2023, the Institute for Medical Research and Occupational Medicine hosted the second "Synergy at the chemistry-nanotechnology interface" symposium, organized as part of the SENDER project (HRZZ-PZS-2019-02-4323) funded by the "Research Cooperability" Program of the Croatian Science Foundation. The organizing committee of the symposium included Ivana Vinković Vrček, Nikolina Kalčec, Nikolina Peranić, and Ivan Mamić. The symposium was opened by SENDER's project leader, Ivana Vinković Vrček, followed by a plenary lecture by Martina Lihter from the Institute of Physics, Zagreb. Apart from her very interesting plenary lecture, short oral presentations on topics in the field of chemistry and nanotechnology were also held by numerous colleagues from the Institute of Physics, the Faculty of Pharmacy and Biochemistry, the Institute for Medical Research and Occupational Medicine, the Faculty of Physics in Rijeka, and the Faculty of Medicine in Rijeka.

2D material-based nanodevices

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The synergy of chemistry and nanotechnology is pivotal in driving the progress of advanced materials and new technologies. A novel class of two-dimensional (2D) materials with atomic thinness showcases unparalleled electronic, optical, and mechanical properties, making them highly versatile in numerous applications ranging from (opto)electronics, sensing, and catalysis to energy harvesting. The incorporation of these materials into nanodevices such as nanopore-based devices and field-effect transistors (FET) unlocks a wealth of new possibilities, particularly in biosensing, sequencing, and manipulating molecules and ions at the single-molecule level. This presentation will focus on the nanofabrication and applications of nanopores (1) and nanopore-FET (2) devices made from 2D materials, including chemical functionalization methods (3, 4), which open immense opportunities in designing novel hybrid materials and tailoring material properties to suit each specific application.

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Strain-engineering of devices based on atomically-thin materials

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In our work, we investigated different segments required for the understanding of physical properties and device implementation of various 2D materials (2DMs) such as MoS_2, borophene, and graphene monolayers as well as MoS_2/graphene heterostructures in terms of flexible electronics. First, different device fabrication procedures were investigated in order to determine the optimum type of 2DM growth, sample morphology, and method of transfer. Several experimental techniques were used: AFM, SEM, Raman and PL spectroscopy, and transport measurements which proved that large-scale monolayers (>100 μm) are advantageous over smaller isolated flakes for devices such as transistors, strain sensors, and photodetectors. The main focus was given to strain-dependent measurements with uniaxial bending of 2DMs, thus enabling the investigation of tensile strain on device performances. We have shown that, on encapsulated devices, strain uniformly propagates over the whole surface of 2DMs, while with a transfer of 2DMs on pre-fabricated electrodes, the electrodes are a source of inhomogeneous strain distribution within the 2DMs. Our constructed devices based on large-scale MoS_2 monolayer exhibited long lifetimes even at strain values as high as 1 % and during more than 40 bending cycles. Additionally, we have demonstrated that the photoresponse of MoS_2 can be significantly enhanced by the application of strain, where the increase of photocurrent and broadening of spectral sensitivity have been confirmed. Overall, our research provides new insight into the physical phenomena behind the synthesis and manipulation of 2DMs for their utilization as novel flexible electronics and optoelectronics.

Boron in the nanoworld: electronic structure of atomically-thin borophene on iridium

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Investigation of the electronic structure of two-dimensional (2D) materials is crucial for their thorough understanding and technological utilization. Borophene, a 2D form of boron, is characterised by metallicity and polymorphism. The metallic character of borophene renders the material a potential conductive layer in future flexible electronics. All polymorphs of borophene, predicted (1) and realised experimentally to date (2) are metals (3). The ability of boron atoms to adopt various geometrical configurations in two dimensions allows for the atomic-scale design of both structure and properties. Although there are published theoretical predictions of borophene stability and structure on different metallic substrates (4), accompanying valence-band and core-level spectroscopy experiments investigating the electronic structure of the pristine epitaxial borophene systems remain lacking judging by the few publications on the subject. The same holds for functionalized borophene samples, which have also been scarcely studied to date. Employing borazine as a precursor, we grew a millimetre-sized borophene monolayer on Ir(111) exhibiting a unique geometry, evidenced by the low energy electron diffraction (LEED) pattern and low energy electron microscopy (LEEM) images (5). We present our results on surface mapping with two *in situ* photoemission techniques, *i.e.* angle-resolved photoemission spectroscopy (ARPES) and core-level spectroscopy which display subtle changes in the electronic structure of Ir(111) after the borophene growth. The obtained spectra enlighten details of relatively weak binding between the borophene monolayer and Ir(111).

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Kick: a stochastic search procedure for locating neutral gold nanoclusters

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In our research, we employed the "kick" stochastic search method to reproduce the Au(n) nanoclusters documented in previous studies. This method stands out for its simplicity and unbiased nature, as it only requires a single click to generate results, eliminating the need for chemical intuition. To assess the performance of the kick method, we compared it with other methods available in the literature, and we achieved favourable results. Using the kick method, we generated geometries for Au(n) clusters with an even number of atoms. These generated structures were subsequently optimized using Gaussian software, employing the M06L and BP86 functionals in conjunction with the LANL2DZ basis set. Through the optimization process, we successfully located all the clusters that had been previously reported in the literature. Moreover, we discovered additional stable structures that had not yet been documented. We conducted our search on Au_4, Au_6, and Au_8 clusters and using the Kick method could locate both minima and higher order stationary points.

In vitro assessment of differently functionalized gold nanoparticles for potential delivery of L-DOPA

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L-DOPA is the most effective drug for Parkinson's disease motor symptoms, but it is associated with significant complications; more than 50 % of patients develop levodopa induced dyskinesia after 6 years of treatment. Dyskinesias – involuntary, erratic movements – lead to difficulties in performing everyday tasks that require fine motor control and significantly reduce quality of life (1). Continuous drug delivery systems, like the infusion of levodopa/carbidopa intestinal gel, can reduce dopamine fluctuations and alleviate dyskinesias (2). However, drug delivery with intestinal gel is complex, invasive, and requires a surgical procedure for a tube placement through which the gel is administered and can lead to complications such as tube dislocation, infections, and abdominal pain (3). This necessitates the development of novel formulations to achieve continuous drug delivery without mentioned problems. Nanocarriers may be a promising approach to Parkinson's disease management by enabling targeted and continuous drug delivery (4). We have developed gold nanoparticles (AuNP) loaded with levodopa and examined its toxicity, uptake, and potential protective effects on an *in vitro* model of dopaminergic neurons. Adamantane (Ad) and peptidoglycan monomer (PGM) coated AuNP, loaded with levodopa, did not display significant toxicity up to a concentration of 100 ppm after 24 h of treatment and were able to reduce oxidative stress induced by levodopa. They demonstrated a poor, but dose-dependent increase in cellular internalization.

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In vivo efficacy of L-DOPA nanoformulations for Parkinson's disease

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Parkinson's disease (PD) is a neurodegenerative disease predominantly affecting the elderly, with no current cure but symptomatic relief provided by medications. The disease manifests in the impairment of cognitive functions, skilled movements, emotions, and memory due to the degeneration of dopaminergic neurons, resulting in a significant reduction in dopamine levels (1). While L-DOPA (LD) stands as the primary treatment, its prolonged usage is associated with serious side effects. This study aims to assess the effectiveness of selenium nanoparticles coated with poly(vinylpyrrolidone) (PVP-SeNPs) and gold nanoparticles coated with peptidoglycan monomer (PGM-AuNPs) as a delivery system for L-DOPA. A hemiparkinsonian rat model, induced by injecting 6-hydroxydopamine into the right hemisphere, was employed to evaluate the efficacy of this novel delivery approach (2). Following a 28-day post-surgery period, rats were treated by gavage. The treatments included LD alone, LD-doped PVP-SeNPs, LD + Carbidopa-doped PVP-SeNPs, PGM-AuNPs combined with LD and LD + Carbidopa. Behavioral tests conducted both before and after treatment aimed to gauge the efficacy of the *in vivo* PD model and assess the impact of the treatments. Results revealed that treatment with LD + Carbidopa-doped PVP-SeNPs exhibited significant improvement in male locomotor behavior compared to the LD + Carbidopa group. Similarly, the administration of PGM-AuNPs in combination with LD and LD + Carbidopa demonstrated notable enhancement in female motor functions compared to the LD + Carbidopa group. These findings suggest potential benefits of the proposed nanoparticle-based delivery systems in alleviating Parkinson's symptoms, particularly when used in conjunction with L-DOPA.

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Mass spectrometry imaging of cardiac alterations in rats after administration of different doxorubicin formulations

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The clinical utility of the cytotoxic drug doxorubicin (DOX) is hindered by its toxicity to non-cancerous tissues, which can result in serious side effects. Cumulative and dose-related cardiotoxicity is the most known side effect of DOX that can lead to cardiac transplantation or even death (1). Nanoformulations, emerging as innovative drug delivery systems, could facilitate site-specific drug accumulation, thereby mitigating the adverse effects of DOX on non-targeted tissues (2). This study employed mass spectrometry imaging (MSI) to explore the impact of various DOX formulations on non-targeted heart tissues. Biochemical alterations in heart cryosections of Wistar rats treated intraperitoneally over 28 days (once weekly) with liposomal (lipoDOX) and poly(lactic-co-glycolic acid)-based nanoformulations (nanoDOX), were compared to those induced by the conventional (convDOX) formulation. The greatest changes in the levels of biomarkers (spermine, acyl-carnitines (Car), ceramides (Cer), and lysophosphatidylcholines (LysoPC)) were observed after treatment with convDOX. However, the changes in LysoPC and Cer expressions were more prominent in females, suggesting inflammatory processes and apoptosis. In contrast to convDOX, neither lipoDOX nor nanoDOX had any effect on LysoPCs expression showing the benefits of DOX incorporation into liposomes or PLGA nanoparticles. Comparing lipoDOX and nanoDOX, treatment with lipoDOX caused an increase in spermine levels and a decrease in the Car, indicating a change in the fatty acid metabolism. Interestingly, only the expression of stearoyl-carnitine (Car 18:0) was increased after lipoDOX treatment. Although treatment with nanoDOX did not affect the levels of spermine and Cer, it caused an increase of long-chain Car in males only, indicating changes in the mitochondrial metabolism which can cause accumulation of reactive oxygen species (ROS) and promote cardiac damage. Overall, this study showed the potential of MSI in effectively discerning the effects of various DOX formulations on non-targeted tissues, underscoring its importance in drug development.

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Surface modification of metal oxides by pulsed vapor phase click reactions

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Click chemistry includes a category of modular and wide in scope reactions for binding together chemical units having suitable linkage groups. According to the definition of Sharpless et al., who first introduced the term in 2001 (1), these reactions are driven by the formation of thermodynamically stable new bonds, often without any by-product. The strategy has been found applications in many fields, such as drug discovery, biology, polymer and material sciences, etc. Click reactions that can take place between solid substrates and reagents in the gas phase are of great interest because they can be used in vapor phase processes for surface modification reactions or building organic-inorganic hybrid films and other nanostructures where fast and high-yielded reactions are required. In these processes, small molecules from the gas phase are introduced as precursors in sequences separated by a purging inert gas. Their advantages include automatization, scalability, uniform coating of various surfaces (even of highly porous substrates) and excellent film thickness control. Most known are the atomic layer deposition (ALD) for the growth of inorganic thin films, and the molecular layer deposition (MLD) for the growth of organic thin films by employing gas organic monomers. Our efforts toward the introduction of click chemistry in ALD/MLD processes will be presented. More particularly, it will be discussed the surface functionalization of metal oxide surfaces, such as zinc oxide and zirconia particles, in combination with gas precursors including propiolic acid and organic azides in a solvent- and catalyst-free approach (2, 3).

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Nanomechanical tool for application in cancer diagnostics and scientific research

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The study of mechanical features of cancer cells and sub-cellular components provides better insight into molecular changes in cancer progression. By determining the nanomechanical properties, it is possible to identify small changes in morphology, binding forces, and surface adhesion which effectively differentiates normal from cancer cells. Atomic force microscopy (AFM) has emerged as a valuable tool in cancer diagnostics offering unique advantages such as direct cell contact and precise force adjustment (1). This method is based on detecting the weak interatomic interaction between a sharp tip and the sample surface. Measurement of mechanical properties such as cell stiffness, adhesion, and elasticity can serve as biomarkers in cancer diagnosis and research. Studies have shown that stiffness distribution for normal tissue is unimodal, while in cases of malignant tumours, tissue stiffness distribution is heterogenous (2). One of the main advantages of AFM is that sample preparation is straightforward, meaning no labelling or staining of tissues is needed before measurement. Besides that, AFM is applicable in cell research for both suspended and adherent cells, simultaneously obtaining information about structure and mechanical properties with nanometer resolution. Also, when combined with other tecniques like confocal fluorescence microscopy, AFM provides multiple complementary information sources, allowing for a comprehensive understanding of cellular structure and activity.

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Application of atomic force microscopy for investigation of nanostructures from human cerebrospinal fluid

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Every biofluid is abundant with nanostructures. Among them, extracellular vesicles (EVs) are intensively investigated for diagnostic purposes due to the connection of their composition to the state of the parental cell or tissue. EVs in human cerebrospinal fluid (CSF) are heterogenous in morphology, which requires advanced and optimised microscopy methods for proper visualisation and characterisation (1). We compared the images obtained by atomic force microscopy (AFM) in air and liquid and recorded the force-distance curves. Imaging of EVs in liquid showed great advantages regarding the preservation of native morphology and biomechanical measurements. EVs were visualised both in their natural environment as biofluid constituents and in a saline solution after their isolation by size-exclusion chromatography. We identified five different shapes of EVs from AFM and developed a computer program to examine individual 3D structures (2). In the future, automatic subpopulation classification by artificial intelligence and attribution of biomechanical properties of EVs will be developed. This work should help estimate the current state of research on morphology and biomechanics of EVs from human CSF and identify the most efficient pathways towards the application of EVs for diagnostics of brain pathologies.

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Atomic force microscopy for characterisation of extracellular vesicles from human cerebrospinal fluid

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Extracellular vesicles (EVs) are nanometric membrane structures secreted from almost every cell and present in biofluids. As the composition of EVs reflects the state of their parental tissue, EVs possess enormous diagnostic/prognostic potential, especially for application in liquid biopsies. Any such further application requires a detailed characterization of EV size, concentration, and morphology. Traditionally, electron microscopy is used for imaging of EVs, but since the samples are observed under high vacuum they can be damaged during sample preparation and also electron beams can damage the EVs. Imaging of EVs in their native form (in liquid) can be achieved only by atomic force microscopy (AFM). Preparation protocols for AFM imaging include mica functionalization and observation of EVs both in their natural biofluid environment or in buffer saline after EV isolation (1). In this work, the focus is to observe the native morphology of EVs in human cerebrospinal fluid (CSF). Therefore, EVs were separated by gravity size exclusion chromatography (SEC) and investigated by AFM in liquid. Enrichment of EVs in early SEC fractions was confirmed by immunoblotting for transmembrane proteins CD9 and effective elimination of contaminants was shown by immunoblot on albumin. The particle concentration and size distribution were determined in different fractions and in the native CFS pool. AFM showed different morphological features of EVs: round, concave, single-lobed, multilobed and flat EV structures. These subpopulations should be further investigated as they could have different biological functions (2).

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Dissolution of colloidal gallium nanoparticles and their antibacterial activity against *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa is an opportunistic premise plumbing pathogen commonly found in ventilation pneumonia, urinary tract infections and cystic fibrosis. It is also one of the multidrug-resistant pathogens, which pose a serious threat to human health. As the efficacy of traditional antibiotics is strongly decreasing, there is an urgent need for new antibacterial strategies, such as antimicrobial metals, metal ions, and metallic nanomaterials. Gallium ions (Ga^{3+}) have proven particularly effective against *P. aeruginosa* bacteria by hindering their Fe^{3+} uptake due to many similarities of both ions, which blocks redox processes and inhibits various enzymes (ribonucleotide reductase, catalase, superoxide dismutase) (1). Recently, even stronger bactericidal activity against *P. aeruginosa* has been shown for elemental gallium nanoparticles (GaNP), which could also better evade bacterial resistance than ionic gallium due to possible additional mechanisms of antibacterial action (2). However, these nanoparticles were in a composite with hydroxyapatite and exhibited a wide size distribution, while we should focus on GaNP alone with well-defined size to better understand their mechanism. Therefore, we used recently developed methods to create monodisperse GaNP with controllable size that form a stable aqueous colloidal solution and investigated their antibacterial activity against *P. aeruginosa* as well as Ga^{3+} ion release due to their dissolution in bacterial culture medium.

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Preparation of smart polymer nanoparticles for induction of apoptosis in cancer cells

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It is well-known that nitroxide radicals can bind reactive oxygen species and modify signaling pathways of oxidative stress in cells. Furthermore, nitroxide radicals have been shown to suppress tumor growth and induce apoptosis in cancer cells (1). Despite chemotherapeutic potential, their usage under *in vivo* conditions is limited due to unspecific accumulation in normal tissues and rapid excretion. We developed nanoparticles containing poly[N-(2-hydroxypropyl)methacrylamide] (PHPMA) and cholesterol with 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) nitroxide radical bound to the polymer carrier by a pH-sensitive hydrazone bond in order to overcome these difficulties. At pH 5, which corresponds to the pH of endosomal/lysosomal compartments of cancer cells, nitroxide radicals are rapidly released from nanoparticles whereas the release of radicals at physiological pH was not significant. As a result, HPMA based nanoparticles containing nitroxide radicals could be used as smart drugs that target affected organs or tissues (2). In this study, based on stated facts, cancer cell line HCT 116 and normal immortalized cell line hTERT GF were treated with our novel pH-sensitive nanoparticles. We monitored cell viability and protein expression of markers involved in oxidative stress and apoptosis to elucidate the mechanisms of action and chemotherapeutic potential of developed nanoparticles. We expected apoptosis induction in cancer cells and no impact on normal cells.

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SYMPOSIUM ABSTRACTS
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N-glycosylation pattern of extracellular vesicles in traumatic brain injury

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Millions of people suffer from traumatic brain injury (TBI) every year. The process itself is biphasic with first phase causing the mostly irreversible mechanical damage and subsequent secondary phase adds to the damage with neuroinflammatory events. During such a neuroinflamatory response, there is possible and likely disruption of the blood brain barrier (BBB) with extracellular vesicles (EVs) playing the important role in exacerbation of the process. EVs are small, membranous and heterogeneous particles secreted from all cell types and they are loaded with DNAs, RNAs, lipids, and proteins. Their surface is rich in different glycan structures responsible for cell to cell interaction. They can regulate immune response after TBI including the activation of peripheral immune cells and moving the glia to the site of injury. Since EVs are loaded with different signalling molecules involved in promotion of trauma, it is of the most importance to characterize their content in order to use them as potential biomarkers. Furthermore, detailed analysis of EVs glycocalyx enables the distinction between different populations of EVs enables more effective diagnostics and eventually a therapeutic agent that can alter and/or cure specific neurodegenerative processes. Another important factor is the fact that EVs offer a tissue-free biopsy. We have analysed EVs N-glycosylation pattern from human cerebrospinal fluid (CSF) samples. Briefly, EVs glycoproteins were denatured and enzymatically deglycosylated. Then, the released and fluorescently labelled N-glycans were analysed by ultra-high performance liquid chromatography based on hydrophilic interactions with fluorescence detection (HILIC-UHPLC-FLR). The obtained chromatogram indicated the presence of different N-linked glycoforms. However, further in-depth glycomic analysis by reversed-phase-liquid chromatography-electrospray ionisation-MS will decipher the exact glycan structures present on the EVs glycoproteins and thus indicate their biomarker potential.

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In vivo biodistribution evaluation of L-glutamic acid-g-p(HEMA) polymeric nanoparticles

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Recently, with increasing use of polymeric nanoparticles in many areas including drug delivery systems, it has brought along the risk assessments of these materials. While evaluating risk assessment, mostly the biodistribution of these nanoparticles by *in vivo* administration is unknown which brings concerns about potential toxicity. The aim of the study is demonstrate that *in vivo* biodistribution potential of L-glutamic acid-g-p(HEMA) polymeric nanoparticle with present a detailing organ distribution by *in vivo* imaging sytem (IVIS Spectrum). The nanoparticles were distributed depending on time in the body for 24 h after intravenous injection and their presences were observed in the organs by *ex vivo* imaging with IVIS spectrum. As a conclusion, L-glutamic acid-g-p (HEMA) polymeric nanoparticles were distributed and excreted from the body and have a potential to be used as a drug delivery systems.ells.

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