

Fourth regional roundtable:

Refractory, process industry, nanotechnologies and nanomedicine

THE BOOK OF ABSTRACTS

Plenary and invited lectures



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PROBING ASTROCYTES WITH CARBON NANOTUBES: IMPLICATIONS FOR TRANSLATIONAL MEDICINE

Vladimir Parpura

Department of Neurobiology, University of Alabama, Birmingham, Alabama, USA

Carbon nanotubes (CNTs) with their unique properties have emerged as a promising material for use in neural prosthesis. We have varied astroglial morpho-functional and proliferative features using diverse forms of CNTs, which were either applied to cells as colloidal aqueous solutes or served as strata for cellular growth.

We have shown that chemically-functionalized water soluble single-walled carbon nanotubes (ws-SWCNTs), when applied to the culture media, were biocompatible and made the astrocytes larger and stellate/mature, changes that were associated with an increase in glial fibrillary acidic protein immunoreactivity (GFAP-ir). Thus, ws-SWCNTs could have more beneficial effects at the site of a traumatic brain injury or spinal cord injury than previously thought; by affecting astrocytes, they could provide for a more comprehensive re-establishment of the brain's computational power.

We have also used films of SWCNTs as retainable strata for the growth of astrocytes in culture. We have shown that SWCNT films of varying thicknesses (10, 30 and 60nm) were also biocompatible for the growth of astroglial cells. The specific amount of coating appeared important as well, as they differentially affected astrocytic growth. Astrocytes plated onto SWCNT films of higher thickness (60 nm) grew bigger and rounder in culture, which was associated with a decrease in GFAP-ir. Additionally, there was a graded increase in the adhesion and proliferation of astrocytes with an increase in the thickness of the SWCNT films. Described changes of astrocytic morpho-functional properties are critical to understand because CNT-coated electrodes have been shown to be advantageous over standard metal electrodes for use as neural implants in brain recording and stimulation *in vivo*. Our data can be used to make predictions/estimates on the amount of coating that might work best for coating the implants.

Taken together these studies demonstrate that CNTs can be used as strata and colloidal aqueous solutes to affect astrocytic growth, thus showing the proof-of-principle for their use in neural prosthesis applications.

3D PRINTING AND BIOFABRICATION IN BONE TISSUE ENGINEERING

Vladimir S. Komlev

*A.A. Baikov Institute of Metallurgy and Materials Science Russian Academy of Sciences,
Moscow, Russian Federation,*

*Institute of Photon Technologies of Federal Scientific Research Centre “Crystallography and
Photonics”, Russian Academy of Sciences, Moscow, Russian Federation*

The present work is targeted to shortening of rehabilitation time and life quality improvement of the patients with bone tissue diseases through implementation of personalized tissue-engineered methodology and technologies.

The first main objective of this work was development and optimization of three-dimensional (3D) printing and biofabrication processes to fabricate novel, innovative composite scaffolds based on metals, calcium phosphates and polymers for bone tissue engineering needs. The ideal scaffold will act as a three-dimensional template which mimics the extracellular matrix of the tissue onto which bone forming cells can attach, multiply, migrate and function.

The second main objective of the present work was to develop the technology for 3D fabrication of osteogenic-based bone repair systems. Hereby, the living cell component or gen constructs is added to the printed scaffold prior to implantation, which increases the effectiveness and the cost-to-benefit ratio of the biomedical treatment.

The third main objective of the present work was focused for the first time a rapid creation of 3D scaffolds under microgravity conditions at International Space Station (ISS). Further advancement in the technology involving the application of living components provides the possibility for rapid fabrication of tissue engineered constructs with complex shape and specific macro- and microstructure. This technology offers an opportunity for rapid assembly and fabrication of tissue-engineered scaffolds based on calcium phosphates and living cells. Design and development of such assemblers and biofabrication tools in space may contribute significantly to the field of tissue engineering.

Overall results of this work provide concrete opportunities to implement scientific innovation and to allow commercialization and clinical application of the innovative biomaterials required in the fields of orthopedics, dentistry, oral and maxillofacial surgery, neurosurgery and oncology.

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OPERATIVE DENTISTRY AND ENDODONTOLOGY WITH CALCIUM SILICATE CEMENTS – *WHAT LIES BEHIND ALL THIS?*

Till Dammaschke

Wilhelms-University Münster, Münster, Germany

Calcium silicate-based cements are derived from the building material Portland cement and present a new group of materials in dentistry, which includes cements such as ProRoot MTA, MTA Angelus and Biodentine, but also MTA products from other manufacturers. They are characterized by both biocompatibility and bioactivity and possess sealing and sometimes dentin-like mechanical properties. Due to the better materials science properties of calcium silicate cements, these materials are an interesting alternative to the conventional, previously used materials based on calcium hydroxide or other cements.

Calcium silicate cements can be used at dentine defects in the area of the tooth crown and root and, due to their biocompatibility and bioactivity, have a positive effect on vital cells of the pulp and the periodontium. They can, for example, induce tertiary dentine formation during indirect capping and promote the formation of hard tissue in direct contact with vital pulp tissue. Calcium silicate cements can therefore offer advantages in indirect and direct capping and - if correctly indicated - contribute to the long-term vitality of teeth. In addition, calcium silicate cements are particularly suitable for covering perforations in the root canal system and as retrograde obturation material in apical surgery. Calcium silicate-based cements are an innovative material for both endodontics and restorative dentistry, which may offer new perspectives in treatment strategies. For this reason, some companies have recently started offering calcium silicate-based root canal sealers.

In this lecture, an overview of the calcium silicate cements available on the market will be given and the possibilities and limitations of these new materials will be demonstrated.

INORGANIC-ORGANIC HYBRID MATERIALS: SYNTHESIS, CHARACTERIZATION, AND APPLICATION

Jovan M. Nedeljković

Institute of Nuclear Sciences Vinča, University of Belgrade, Belgrade, Serbia

A new class of hybrid composite materials that physically integrate metal-oxide nanoparticles (TiO_2 , CeO_2 , Al_2O_3 , *etc.*) whose optical properties can be finely tuned with small colorless organic molecules, mainly benzene derivatives, is developed. The synthesized inorganic-organic hybrids are thoroughly characterized by microstructural techniques (TEM, XRD, nitrogen adsorption-desorption isotherms), and a variety of spectroscopic techniques (UV-Vis, FTIR, photoelectron spectroscopy). The deeper insight into the optical properties of prepared hybrids is obtained by density functional theory (DFT) calculations using two approaches: (i) the periodic boundary conditions, and (ii) finite-size cluster method. Special attention was paid to the potential practical use of synthesized inorganic-organic hybrids in eco-friendly technologies. The photocatalytic abilities of hybrids were tested in water splitting reaction and degradation of organic dye molecules. On the other side, the antimicrobial performance of synthesized hybrids was evaluated against gram-negative and gram-positive bacteria, as well as fungi.

REFRACTORY TODAY: AN EXAMPLE OF EXTRAORDINARY COOPERATION OF ONE INNOVATIVE AND ONE SMALL MANUFACTURING COMPANY

Jokanović V.¹, Stević M.², Stević D.²

¹ *ALBOS d.o.o.*, ² *REAL S d.o.o.*

The recent trends in refractory on the global level show increase their consumption. In the 2017 this consumption was about 30.6 million tons, while the global projected market before corona virus shows that their consumption could reach 43.7 million tons in the end of 2026, with an increase of 4.1% per year for the period (2018-2026). In terms of value, the global market is expected to reach 41.1 billion dollars by 2026, showing an annual growth of 4.2%. Growth in India is expected will be more pronounced achieving 5.4% its increase. Recently significant increase of consumption of refractory products is present in Asian countries such as China, Japan, India, Korea and ASEAN (Association of Southeast Asian Nations, with its center in Jakarta).

The demand for increased consumption refractory materials is induced by the growth of capital investments and large projects in the sectors of infrastructure, steel, mining (which affects the metallurgy sector) and cement industry and the development of distribution and sales network of refractory materials in the region. The higher demands for refractories is also expected in the glass industry to 2026. As it is well-known the highest demands for refractory products are in the process of production of steel, iron, nonferrous metals, glass, cement and other industries. China and Europe are important regions in the market of the final refractory and steel products on the global and regional level. In addition, the high dependence of refractory manufacturers around the world on imports of raw materials from China already represents a challenge to the growth of the market for refractory products globally. The dominant growth of monolithic and other unshaped materials to a value of about 50.2% of the total market share in terms of production volume by 2026 is expected that in the near future.

This trend points the importance of the technology innovation in refractory industry, placing them in the foreground. The relationship in which is small refractory factory, like REAL S is in close relationship with excellent innovative small company, like ALBOS show extraordinary benefit for both company. For only several years their portfolio particularly in the area of unshaped products become comparable with portfolio large global companies, showing capabilities to make them very tough concurrence in surrounding market, because the properties of refractory products obtained through this

cooperation, the most often design materials with better exploitation characteristics and more favorable price. This cooperation increase sale of materials several times, and it is expected that this year it can reach extremely high values. In addition, this collaboration gives results even on the international level, through cooperation with refractory companies in China, Turkey, Romaniaetc.

CHARACTERIZATION OF THE ELECTRODEPOSITED Zn-Mn-Al₂O₃ COMPOSITE COATINGS

Milorad Tomić¹, Marija Riđošić¹, Miomir G. Pavlović¹, Mihael Bučko², Jelena B. Bajat³

¹*University of East Sarajevo, Faculty of Technology, Zvornik, Republic of Srpska, Bosnia and Herzegovina*

²*University of Defense, Military Academy, Belgrade, Serbia*

³*Faculty of Technology and Metallurgy, University of Belgrade, Belgrade, Serbia*

The recent high industrial demand for more durable and environmentally acceptable finishes has led to development of novel composite coatings with enhanced properties compared to traditional coatings such as Zn and its alloys. Metal matrix composite coatings are composed of the metal matrix and reinforcement particles as secondary inert phase. A number of methods are developed for MMC production, such as powder metallurgy and electrolytic routes. The electrolytic routes are preferable due to low equipment costs and easy process control. Alumina particles have been used extensively in metal-matrix composite coatings, because it possesses excellent chemical stability and good mechanical properties, such as high microhardness and wear resistance [1-3]. The electrodeposition of Zn-Mn-Al₂O₃ is performed galvanostatically from chloride bath. The influences of the deposition current density as well as bath composition on the properties of the composite coating were studied. The morphology is analysed by scanning electron microscope and the chemical composition is analysed by electron dispersive spectroscopy. The corrosion resistance of the electrodeposited composite coatings was analysed by two electrochemical methods, the Tafel polarization method and the electrochemical impedance spectroscopy. Based on the obtained results it was found that manganese content in the coatings decreases with the increase of deposition current density. The coatings deposited with higher current densities are more compact, and defects are less noticeable. The alumina particles were homogenous dispersed in the coatings. The corrosion tests showed that Zn-Al₂O₃ had the lowest corrosion stability in 3 wt% sodium chloride and presence of manganese is beneficial to anticorrosion properties. The composite coatings deposited at 2 Adm⁻² had the lowest corrosion rate.

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INTERACTION OF ELECTRONS WITH CARBON NANOSTRUCTURES AND METALIC SURFACES

Duško Borka

Department of Theoretical Physics and Condensed Matter Physics (020), Vinča Institute of Nuclear Sciences, University of Belgrade, Belgrade, Serbia

In this lecture focus will be given on the theoretical description of electron interaction with carbon nanostructures (graphene and graphite) and different metallic surfaces. It is also investigated graphene supported by metallic surfaces. The electron interaction with material appears to be fundamentally different from the charged ion interaction. The main difference is that electrons do not change their charge state, and clear experimental distinction between the original projectiles (primaries) and secondary electrons generated with the interaction of primaries with the surface is not possible. Theoretically, it is possible to calculate, identify and follow up all electron trajectories. Monte Carlo simulations were performed based on the Classical Transport Theory model to mimic the experimental spectra. We take into account both elastic and inelastic collisions of electrons by atoms during the simulations. For the case of the elastic scattering we use the static field approximation with non-relativistic Schrödinger partial wave analysis. The electron energy loss distributions for primary and for secondary electrons were calculated using the bulk and the surface dielectric functions. We also compared our theoretical results with available experimental data. There is found satisfactory agreement between measured and calculated electron spectra.

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INFLUENCE OF DEFECT CHEMISTRY ON THE MECHANICAL AND FUNCTIONAL PROPERTIES OF MAYENITE ($\text{Ca}_{12}\text{Al}_{14}\text{O}_{33}$)

Branko Matovic

Vinča Institute of Nuclear Sciences, University of Belgrade, Belgrade, Serbia

The binary compounds of the calcia–alumina ($\text{CaO–Al}_2\text{O}_3$) system are significant in a wide range of applications in metallurgical slags, ceramic materials, and cement technology. However, intermediary phase mayenite ($12\text{CaO}\cdot 7\text{Al}_2\text{O}_3$) become one of the more interesting advanced ceramic material. It stimulated research because of its oxygen mobility, ionic conductivity, high anti-carbon and anti-sulfur characteristics and catalytic properties. In this work Mayenite has been prepared using various methods: ceramic route, by solid state reaction between CaCO_3 and $\gamma\text{-Al}_2\text{O}_3$, by modified glycine/nitrate procedure and by crystallization from the melt. Also, room-temperature stable Electride $[\text{Ca}_{24}\text{Al}_{28}\text{O}_{64}]^{4+} (\text{e}^-)_4$ was obtained from obtained insulator powder with composition corresponding to phase Mayenite by heating in reducing atmosphere. The free oxygen ions (O^{2-}) from crystal structure of mayenite can be removed via appropriate reduction treatments. In this work, the sample powder was set in a carbon crucible and was heated at $900\text{ }^\circ\text{C}$ in a flowing N_2 atmosphere. The thermal treatment caused the color change from white to dark green. The reduction process was replaced free O^{2-} ions with electrons converting insulating phase ($12\text{CaO}\cdot 7\text{Al}_2\text{O}_3$) into conductor. The powder X-ray diffraction confirmed that the both insulating and conductor mayenite phase are the same indicating that the reduction treatment does not change the crystal structure. The obtained powders were characterized by Scanning electron microscopy (SEM), Raman spectroscopy at room temperature. A relative density of 94% is obtained at $1300\text{ }^\circ\text{C}$. The photoluminescence spectra measurements were done under continuous Nd YAG laser excitation at 532nm. Data were collected from Ocean Optics USB2000 (200-800 nm) spectrometer. High-pressure luminescence was measured in a Betsa high pressure membrane diamond anvil cell up to 23 GPa with steel gasket (T301). Sample chamber was $125\text{ }\mu\text{m}$ in diameter and $70\text{ }\mu\text{m}$ in thickness. A mixture of methanol and ethanol with a 4:1 volume ratio was selected as the pressure-transmitting medium. Ruby loaded with sample determine the pressure. For measuring Ruby R1 line shift HR2000 Ocean Optics spectrometer (600-800 nm) was used. The effect of doping concentration on

photoluminescence properties of Eu^{3+} -doped mayenite were studied and are discussed. With the increasing of Eu^{3+} doping concentration, the red emitting intensity exhibited a behavior that increased firstly and then decreased. The optimal Eu^{3+} ion concentration is found to be 1.5%. Obtained results suggest that $\text{C}_{12}\text{A}_7:\text{Eu}$ phosphor may serve as a promising red luminescent materials used in fabrication of optical storage and illumination in dark environments.

MAGNETIC IRON OXIDE NANOPARTICLES AS DIAGNOSTIC AND THERAPEUTIC AGENTS

N. Jović Orsini¹, M. M. Milić¹, B. Babić-Stojić¹, D. Milivojević¹, M. Požek², V. Jokanović

¹*Vinča Institute of Nuclear Sciences, University of Belgrade, Belgrade, Serbia*

²*Department of Physics, Faculty of Science, University of Zagreb, Zagreb, Croatia*

³*ALBOS d.o.o.*

Due to their unique magnetic properties, iron oxide nanoparticles (IONPs) are important items for the development of advanced biomedical applications and products, which can serve in cancer therapy, diagnosis, biosensing, etc [1]. The ability of IONPs to respond to an external magnetic field makes them stimuli-responsive nanosystems. Such as, they can be used for diagnosis, mostly as a contrast agent in magnetic resonance imaging (MRI), and recently also in magnetic particle imaging (MPI). Their therapeutic applications include advanced cancer treatment, such as magnetic fluid hyperthermia (MFH) and magnetic drug targeting.

Thanking to *magnetotactic bacteria*, nature is already able to produce nanoparticles consisting of magnetite (Fe₃O₄) core surrounded by a phospholipid bilayer, called *magnetosomes*. Magnetosomes are characterized by excellent crystallinity, a well-defined morphology, low toxicity and good stability. They are considered to be among the best agents for MFH and MRI. Contrary to nature, we (human beings) try to produce synthetic IONPs, which will possess similar properties as magnetosomes.

Here, we summarize our recent progress in the design and synthesis of magnetic IONPs. One of the investigated systems is a ferrofluid made of ultra small maghemite nanoparticles which could be used as a positive, T₁-contrast agent for MRI [2]. We also provide our recent results concerning fabrication of single crystalline Fe₃O₄-based nanoparticles with high heating ability for MFH application [3].

SIGNIFICANCE AND APPLICATION OF NANOPARTICLES IN ENDODONTICS

Bojana Ćetenović

Vinča Institute of Nuclear Sciences, University of Belgrade, Belgrade, Serbia

Bacterial persistence of the root canal is considered one of the main reasons for the failure of endodontic therapy. Due to the absence of oxygen and lack of nutrients, bacterial species detected in the infected root canals are restricted. Nevertheless, biofilm was observed in the apical third of more than 70% of endodontically treated teeth with apical periodontitis. Therefore, the main goal of endodontic therapy is the elimination of biofilm, since the presence of vital microorganisms at the moment of root canal obturation is considered the most crucial for the therapy success. Conventionally used antimicrobials during mechanical instrumentation, ethylenediaminetetraacetic acid (EDTA) and sodium hypochlorite (NaOCl), showed some limitations in achieving effective root canal disinfection. So many studies have been focused on the development of antimicrobials that will overcome existing problems.

Application of nanoparticles showed promising therapeutical effects due to ultra-small size, large surface-to-mass ratio and increased chemical reactivity. Nanoparticles of silver (AgNPs) may provide a new approach for root canal irrigation. The large surface area and high charge density of AgNPs allow interaction with the negatively charged surface of bacterial cells resulting in enhanced antimicrobial activity.

Many investigations have also been focused on finding new formulations to improve physicochemical disadvantages of retrograde filling materials. Combination of fully innovative sol-gel method with high-temperature self-propagating reaction may decrease the setting time of material and enhance its biological properties. New nanostructured endodontic material based on mineral polyoxide carbonate aggregate, synthesized by using this method, showed increased biocompatibility and bioactivity compared to mineral trioxide aggregate, and may be recommended for further clinical investigations.

INSIGHT IN THE METHODOLOGY OF THE BIOLOGICAL EVALUATION OF NOVEL BIOMATERIALS

Zogovic N.¹, Tovilovic-Kovacevic G.², Despotovic A.¹

¹*Department of Neurophysiology*

²*Department of Biochemistry*

Institute for Biological Research "Siniša Stanković" – National Institute of the Republic of Serbia, University of Belgrade, The Republic of Serbia

Biomaterials represent materials that are entities, surfaces or constructs that interact with specific biological systems. The use of biomaterials dates from ancient times when naturally derived materials were used in an attempt to structurally replace tissues lost to disease or trauma. Significant progress in biomaterials science and engineering, in the mid-1940s, led to the replacement of naturally derived materials with synthetical materials that possess better performance, increased functionality, and more reproducibility. An adequate and successful application of biomaterials requires evaluation of its biological response. Biocompatibility or biological response means an ability of a material to perform its intended function without any harmful effects either locally, in the area of implantation, or for other tissues, organs, or organism. The evaluation of biological response is very complex and specific; depends on the unique properties of the material and its intended role in the biological system. The biomaterials must be blood compatible, nontoxic (unless it is their intended function), non-inflammatory, non-pyrogenic, non-allergenic, and non-carcinogenic. To assess these requirements a set of methods are foreseen which can be roughly divided into *in vitro* and *in vivo* tests. The starting point of a biological response evaluation is the measurement of the cytotoxic potential of leachable or secreted substances from biomaterials to cells *in vitro*. Changes in intracellular signalling pathways observed in presence of biomaterial should be considered carefully, also. If the lack of biomaterial toxicity *in vitro* has been established, further testing should be directed toward the validation of its biocompatibility *in vivo*. The next steps in the evaluation of biocompatibility *in vivo* include assessment of the immune response of surrounding tissues to the biomaterial, as well as estimation of acute and subchronic systemic toxicity after material implantation. For an implant, a desired biological response *in vivo* is a mild

inflammatory reaction, which resolves in a generation of a thin, fibrous capsule on a biomaterial with no active or progressive reaction, locally or systemically. In addition, when evaluating a biological response, it should always bear in mind that biocompatibility is a dynamic process since both material characteristics and host response are changing over time due to aging, disease, etc. Therefore, special attention should be given on time-dependent investigation of biomaterials intended for prolonged and permanent body contact. The progress in material science and engineering will allow the design of more sophisticated biomaterials with increasingly complex properties, which will undoubtedly present a challenge for biocompatibility assessment of new materials.

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CALCIUM ALUMINATES: COULD THEY BE PROMISING ALTERNATIVE FOR CALCIUM SILICATE IN DENTISTRY?

Djordje Antonijević

Vinča Institute of Nuclear Sciences, University of Belgrade, Belgrade, Serbia

Calcium silicates are widely used in restorative, endodontic and surgical clinical procedures for the following clinical applications: pulp capping, pulpotomy, orthograde/retrograde root canal filling, root canal perforation repair, apexification and apexogenesis. In recent years, calcium aluminates have been introduced to be an alternative to calcium silicates in dentistry. This work debates the advantages and downsides of calcium aluminates as compared to calcium silicates for dental application. Calcium aluminate-based cements are composed of calcium aluminate and calcium dialuminate phases. The main advantages of calcium aluminates are their superior physical properties, primarily higher compressive strength. Biological effect of calcium aluminates is documented to be satisfactory both *in vitro* and *in vivo* (tested in subcutaneous tissue of rats and rats' tibia). Long term toxicity investigation in rat's liver shows that it does not have adverse effects. They are proven to have lower solubility if enriched with adequate radiopacifying agents. Similar to calcium silicates, calcium aluminates are capable to precipitate the crystals on their surfaces when soaked in simulated body fluid, proving its bioactivity. Although calcium aluminates increase the pH value of the solution, it shows lower values compared to calcium silicates due to lower release of calcium ions. Antimicrobial activity of calcium aluminates is weaker compared to calcium silicates. Addition of silver to its composition may increase its antimicrobial feature. Regarding the influence of calcium aluminates on tooth color, it results in similar alterations (ΔE), but luminosity change (ΔL) is lower than in MTA. In conclusion, calcium aluminates present suitable alternative to calcium silicates in dentistry and have numerous advantages, primarily from physical standpoint. However, the higher amount of aluminum in their composition remains the challenge from biological point of view. Thus, it is worthwhile to investigate chronic toxicity of calcium aluminates before their clinical application.

CHALLENGES AND OPPORTUNITIES OF NANOSTRUCTURAL BIOMATERIALS IN ENDODONTICS

Marijana Popović Bajić.

Faculty of Dental Medicine University of Belgrade, Belgrade, Serbia

Nanotechnology is one of the most intriguing disciplines of modern science research. It is based on molecular self-organizing and new material creation with dimensions imitating the zero dimension. Today it is implemented in various science fields.

Calcium-silicate and calcium-phosphate ceramics in combination with hydroxyapatite-based nanomaterials are used as basic biological substitutes in regenerative medicine. They are used in reparation, sustaining or improving the damaged calcified tissue. Growth of new scaffold in regenerative endodontics is a new field of research of dental biomaterial. The results of experimental research on cell cultures and different experimental models have shown high level of biological performance of the newly synthesized materials.

Calcium-silicate and calcium-aluminate based materials are still used in endodontic therapy with diseases where success is still unreliable (direct pulp capping, apexogenesis, pulp regeneration), as well as inter-radicular perforation and root canal obturation therapy.

Experimental research with these nanomaterials on cell culture by subcutaneous tissue implanting, direct pulp capping of rat, rabbit, swine and sheep teeth have showed pronounced bioactive characteristics and dentine bridge formation with structural characteristics resembling those of natural dentine. A positive bio response of tissue to the application of these materials presents a good foundation for possible modifications in their composition, as well as clinical investigation and research which will raise the efficiency and longevity of certain therapeutic procedure.

EFFECTS OF BETHAMETASONE ON HEMATOLOGICAL PARAMETERS OF *RATTUS NORVEGICUS* BERKENHOUNTE 1769 RATS, IN *IN VIVO* CONDITIONS

Edhem Hasković

Faculty of Science, Department of Biology, University of Sarajevo, Sarajevo, Bosnia and Herzegovina

Corticosteroids are a class of steroid hormones secreted by the adrenal cortex, which are broken down into mineralocorticoids and glucocorticoids. In addition to affecting carbohydrate metabolism, glucocorticoids regulate other processes such as cell growth and development, metabolism, maintenance of homeostasis and suppression of the immune response, which is why they are widely used as therapeutic agents against a wide variety of diseases including inflammation, autoimmune diseases and cancers. Betamethasone (16 β -methyl-9 α -fluoroprednisolone) is a long-acting synthetic fluorinated glucocorticoid with metabolic, immunosuppressive, anti-inflammatory and antiproliferative effects, most commonly used for topical use in the treatment of dermatoses. In this paper, we monitored the effect of betamethasone on the total leukocyte count and percentage of individual leukocyte counts, erythrocyte count, hemoglobin concentration, hematocrit, and hematologic indices (MCV, MCH, and MCHC) using hematological parameters using adequate methods. The effect of betamethasone on a total of 10 individuals administered betamethasone at different concentrations was examined. The first experimental group consisted of five individuals administered betamethasone at a concentration of 0.0002 mg / g body weight. The second experimental group also counted five individuals administered betamethasone at a concentration of 0.0004 mg / g body weight. By comparing the hematological parameters of the two groups, a significant increase in the total leukocyte count ($p = 0.00$) of the individuals of the other experimental group was observed. Regarding the differential blood count, a significant increase in the proportion of segmented ($p = 0.00$) and non-segmented neutrophils ($p = 0.00$) was observed, as well as a significant decrease in the lymphocyte ($p = 0.00$) and monocyte ($p < 0.05$) proportions in individuals of the second group a higher concentration of betamethasone was administered than the subjects of the first experimental group, four hours after administration. Betamethasone administration showed no significant change in the eosinophil and basophil representation ($p > 0.05$). A significant increase in erythrocyte counts ($p = 0.00$), hemoglobin concentration ($p = 0.00$) and hematocrit values ($p = 0.00$) was observed in individuals in the second

experimental group who were administered higher concentrations of betamethasone, which is consistent with most studies to date. Betamethasone had no significant effect on hematologic indices of MCV, MCH, MCHC ($p > 0.05$).

KINETIC EFFECTS OF CORTICOSTEROIDS ON ENZYME ACTIVITY *IN VITRO*

Safija Herenda

Faculty of Science, Department of Chemistry, University of Sarajevo, Sarajevo, Bosnia and Herzegovina

Most pharmaceutical and nutritional compounds are marketed as enzyme inhibitors and such inhibitors show their specific action in inhibiting enzymes within cells. Since their discovery, corticosteroid treatment has been one of the most commonly used and effective treatments for various inflammatory and autoimmune disorders. Corticosteroids have anti-inflammatory and immunosuppressive effects, so it is hypothesized that betamethasone will have an inhibitory effect on the enzyme catalase. The aim of this study is to examine the effect of corticosteroids betamethasone dipropionate on catalase activity *in vitro*. At an optimal pH of 7 and a temperature of 37°C, the activity of catalase is lower, the higher the concentration of fluoride, ie betamethasone dipropionate. For a living organism, this means more hydrogen peroxide and a greater likelihood of developing free radicals and damaging them. This conclusion is supported by the fact that long-term use of corticosteroids leads to a variety of side effects, often with severe forms of illness, which were primarily treated with this type of medication. A spectrophotometric method based on the reaction between hydrogen peroxide, enzymes and corticosteroids was used. After incubation at 37°C, the addition of ammonium heptamolybdate rewarded the stable complex and analyzed the signals. The results obtained indicate an acompetitive type of inhibition. As the concentrations of betamethasone in the reaction system increase, so does the progressive decrease in the values of the constants V_{max} and K_m , suggesting that this is an acompetitive type of inhibition. Inhibition of catalase suggests that decomposition still occurs in part after betamethasone inhibited the enzyme-substrate complex, or the rewarded enzyme-substrate-inhibitor complex dissociates, in part, into the enzyme, product, and inhibitor. The highest percentage of inhibition was present at a betamethasone dipropionate concentration of 33.81 μM .

TiO₂ NANOSTRUCTURES MODIFIED FOR IMPROVED PHOTOCATALYTIC PURIFICATION OF WASTE WATER

Andreja Gajović¹, Milivoj Plodinec², Vedran Kojić¹, Tihana Čižmar¹, Ivana Panžić¹, Salamon Krešimir¹,
Lucija Radetić³, Marc Willinger⁴, Miran Čeh⁵, Ivana Grčić³

¹*Ruđer Bošković Institute, Zagreb, Croatia*

²*Fritz-Haber-Institute der Max-Planck-Gesellschaft, Berlin, Germany*

³*University of Zagreb, Faculty of Geotechnical Engineering, Varaždin, Croatia*

⁴*ETH Zürich, Zürich, Switzerland*

⁵*Jožef Stefan Institute, Ljubljana, Slovenia*

Increasing awareness of harmful consequences of persistent organic pollutants in water makes development of cheap and stable photocatalysts for their degradation one of significant goals in an environmental engineering. Materials based on nanostructured TiO₂ are among the most prominent materials for the last 40 years. However, one of the main limitations for the application of TiO₂ in photocatalysis is a wide energy bandgap ($E_g = 3.2$ eV for anatase, 3.02 for rutile), so its application is limited to UV light radiation. In order to increase the efficiency of the TiO₂ nanostructures for the photocatalysis, it is essential to expand the spectral range in which the photocatalyst is active, and to prolong excited electron-hole pairs lifetime. Therefore, the strategies for the improvement of their photocatalytic activity in the UV light region as well as broadening of their photocatalytic activity in the visible and near IR light were studied. The photocatalytic degradation of water pollutant by using TiO₂ nanotubes arrays (NTA) with surface modification will be presented.

The synthesis of TiO₂ NTA was obtained by anodization of titanium foil in organic electrolyte. Different approaches to modifications of the synthesized TiO₂ NTA were applied. Surface decoration of NT with Ag nanoparticles was done by photo-reduction, decoration of NT with FeOOH/Fe₂O₃ was done by hydrothermal synthesis, while with Cu-based compounds by spin-coating of copper acetylacetonate. In some cases, the additional modification of the NT surface was done by processing in reduction atmosphere. The structure of photocatalysts were investigated by X-ray diffraction (XRD), Raman spectroscopy, scanning and transmission electron microscopy (SEM and TEM), while the

photocatalytic degradation was studied using model pollutants that present real waste in drinking water as serious industrial and pharmaceutical pollutants; 1H-benzotriazole and salicylic acid.

It was shown that the photocatalytic activity of studied nanostructures was considerably increased by optimization of the TiO₂ bandgap to obtain absorption in the visible and near-IR regions of the solar irradiation. That was achieved by decoration with Ag, FeOOH/Fe₂O₃, or Cu-based compound nanoparticles, and/or due to the defects obtained in the reduction atmosphere. Thus, shift of the photocatalytic activity in the visible and near-IR regions of the spectrum would allow the work of photocatalytic reactors for purification of the water using only sun light without appliance of additional UV lamps. The photocatalytic properties of the synthesized photocatalysts will be discussed considering the morphology, crystal structure and influence of modification and decoration.

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STRUCTURAL INTEGRITY OF MODEL LIPID MEMBRANES WITH INSERTED FLAVONOLS UNDER OXIDATIVE STRESS CONDITIONS

Anja Sadžak¹, Nadica Maltar Strmečki¹, Goran Baranović², Janez Mravljak³,
Zoran Arsov⁴, Jan Pribyl⁵, Manfred Kriechbaum and Suzana Šegota¹

¹*Division of Physical Chemistry RuđerBošković Institute, Zagreb, Croatia*

²*Division of Organic Chemistry and Biochemistry, RuđerBošković Institute, Zagreb, Croatia*

³*University of Ljubljana, Faculty of Pharmacy, Ljubljana, Slovenia*

⁴*Jožef Stefan Institute, Ljubljana, Slovenia*

⁵*CEITEC, Masaryk University, Brno, Czechia*

⁶*Institute of Inorganic Chemistry, Graz University of Technology, Graz, Austria*

Flavonoids, polyphenolic biomolecules with antioxidative activity, have recently emerged as potential novel therapeutics for neurodegenerative diseases. The research of the flavonoids and their use for diagnostic and therapeutic purposes have created a need for understanding the complex interactions of cells with flavonoids, particularly neuroprotective ones like myricetin and quercetin (aglycones) and myricitrin (glycoside). Membrane-flavonoid interactions are of crucial importance for both the cell uptake and toxicological investigations. The presence of flavonoids embedded within a lipid bilayer, can lead to changes in lipid packing by modifying interactions amongst the lipid headgroups and/or acyl tails. Therefore, investigation of flavonoids interaction with lipid membranes using electron paramagnetic resonance, FTIR spectroscopy, atomic force microscopy (AFM), force spectroscopy (FS) and small angle X-ray scattering (SAXS) have been undertaken.

Model membranes consisting of unsaturated phosphatidylcholine DOPC was chosen enabling investigation of not only the nanomechanical properties of model membranes and their interactions with different molecular species, but also testing of flavonoid ability to inhibit lipid peroxidation and lipid structural reorganization in model membranes and neurons under oxidative stress in vitro.

Our research deciphered interactions of neuroprotective flavonols, quercetin, myricetin and myricitrin with model lipid membranes through their delicate response at physiological and oxidative stress environment. Obtained results pointed that the position of the inserted flavonol within the model

membrane (that depends on the hydrophobicity of the flavonol) needed might be considered as the measure of flavonoid protective power, particularly the membrane structural integrity. The nanomechanics (elasticity and fluidity), surface topography (roughness) of model lipid membranes as well as the thermotropic properties that result from the oxidative damage are quantified for the first time showing specific effects on the structural integrity of the membrane at both physiological and oxidative stress conditions.

RETZIUS NERVE CELLS OF THE LEECH AS A MODEL FOR INVESTIGATION OF NEURAL ELECTROPHYSIOLOGICAL EFFECTS OF NANOPARTICLES

SrdjanLopicic and SvetolikSpasic

Institute for Pathological Physiology, School of Medicine, University of Belgrade

Leeches, although being invertebrates, represent a very useful model for research of electrophysiological effects of various stimuli on nervous system for a number of reasons. Leeches have served as an important experimental preparation for neuroscience research since the late 19th century and, therefore, their nervous system has been thoroughly studied and all major characteristic are well defined. The leech nervous system is relatively simple and readily accessible allowing for relatively easy preparation of a variety of semi-intact preparations with preserved interneuronal connections, glia-neuron crosstalk and relations to the periphery, features lacking in many of the more sophisticated models. The semi-intact preparations could have special significance in terms of nano-sized materials as they potentially allow formation of the biomolecular corona which can affect not only the targeting ability of nanoparticles, but also particle size, stability, and overall surface properties. In context of electrophysiology, intracellular neuronal activity can be recorded readily because the somata are relatively large and visible, most relevant electrical parameters can be measured, and intracellular recordings reveal relatively large individual synaptic and action potentials. Finally, leeches are a cheap model which fits well with the 3R ethical requirements, making their use beneficial both in financial and paperwork terms.

More reasons for use of this long-neglected model will be presented, as well as some of our own results of electrophysiological research of nanoparticle effects.

DERIVING ATOMIC MODELS OF DETERGENT MICELLES BY COMBINING SIMULATIONS AND EXPERIMENTAL DATA

Miloš T. Ivanović¹ and Jochen S. Hub²

¹*University of Zurich, Switzerland*

²*Saarland University, Germany*

Small-angle X-ray scattering (SAXS) is an increasingly popular experimental technique used to study biomolecules under near-native conditions. As the information content of the experimental data is low, methods that integrate the data with accurate computational models are urgently required. In recent years, all-atom molecular dynamics (MD) simulations have developed to an increasingly popular and reliable tool for interpreting SAXS experiments and, more general, for gaining atomic-level insights into biomolecules.

Detergent micelles are often employed as a membrane mimics to solubilize membrane proteins. Accurate information about the size and shape of micelles is important for the stability of protein detergent complex. We show how MD can be combined with the SAXS data to derive temperature dependent atomic models of micelles. In the next step, we derive an ensemble of detergent micelles in agreement with experimental data, enabling us to study the influence of various effects on SAXS curves and thereby making an important step towards the better understanding of the SAXS experimental data.

NEW HORIZONS IN BONE AUGMENTATION

Vladimir Biočanin^{1,2}

¹Clinic for Oral Surgery, Faculty of Stomatology, Pančevo, Serbia;

²Clinic for Oral Surgery Faculty of Pharmacy and Health, Travnik, Bosnia and Herzegovina

Modern dental implantology requires sufficient and stable bone and soft tissue around dental implants. In some situations, there is lack of bone for implant insertion. In such situations it is advisable to augment bone before implant insertion. Nowadays, there are different ways for bone augmentation in dentistry. Alveolar socket preservation, guided bone regeneration, sinus lift, filling intra-bony defects, widening of alveolar ridge, present indications for bone augmentation in dentistry practice. Bone substitute nano-particles enable current trend toward faster and better bone healing. In addition, bone substitutes may be mixed with different organic parts, and medicines with controlled delivery. Tissue engineering seeks to combine the bone graft substitutes to growth factors to provide structural support and promote more rapid bone healing. Bone tissue engineering enables osteogenic differentiation of stem cells into 3D bone models. Scaffold in tissue engineering helps migration, proliferation, and differentiation of osteogenic cells promoting regeneration of new bone. For that reasons, scaffold must be stable, biocompatible, biodegradable, and also, porous for cells ingrowth and vascularization. Current advances in 3D printing enable creation of site-specific 3D bone implants for the treatment of critical-size bone defects. Perfect fitting of 3D bone models to bone defects allows faster bone healing with perfect esthetic results.

EFFECT OF ALBO-OS ON BONE REGENERATION: A HISTOLOGIC AND HISTOMORPHOMETRIC EVALUATION

Sanja Milutinović-Smiljanić

Department of Histology, Faculty of Dental Medicine University of Belgrade, Belgrade, Serbia

Hydroxyapatite coated with poly-lactide-co-glycolide (Albo-Os) is synthetic biomaterial developed as an alternative to the autogenous bone grafts and xenografts. The aim of the present study was histologic and histomorphometric evaluation of the Albo-Os effect on bone regeneration in two experimental models in rabbits. Calvarian defects measuring (6 mm) were made in 19 rabbits. Defects were separately filled with Alboos and Bioos and empty defects were revealed as negative control. Histological and histomorphometric analysis were performed in animals sacrificed after 3, 6 and 9 weeks. Also 2.2 cm rabbit's ulna was reconstructed with three dimensional bone construct manufactured from the Albo-Os using models derived from the computed tomography scanning of rabbit's ulna and gradient 3D printing of the bone substitute. After 12 weeks of the implantation, rabbit was sacrificed and defect region was characterized histologically. Three weeks after the implantation in calvarian defects there were trends toward increased healing in Albo-Os group than in Bio-Os, but the difference in new bone formation and inflammatory cells number was not statistically significant ($p > 0.05$). However, 6 weeks and especially 9 weeks after materials' implantation in calvaria superior healing was observed in Albo-Os than in Bio-Os ($p < 0.05$). The results of histological analysis of rabbit's ulna revealed new bone formation with both endochondral and endesmal type of the ossification. Histomorphometric analysis showed that there was no statistical difference between central and peripheral part of the defect neither for new formed bone ($35 \pm 11\%$ vs. $51 \pm 11\%$, respectively) nor for surface of mineralized bone measured ($35 \pm 11\%$ vs. $49 \pm 11\%$, respectively) ($p > 0.05$). Neither connective tissue nor construct particles were observed in the peripheral part of the defect. Based on our findings higher bone formation was detected in calvarian defects filled with Albo-Os

compare to Bio-Os. Also Albo-Os possesses great potential in segmental osteotomy reconstruction.

MECHANISMS OF CALCIUM PHOSPHATES FORMATION ON TiO₂ NANOMATERIALS. A BIOMIMETIC SYNTHETIC ROUTE FOR MULTIFUNCTIONAL NANOCOMPOSITES FOR HARD TISSUE REGENERATION

Ina Erceg¹, Atiđa Selmani¹, Andreja Gajović¹, Ivana Panžić¹, Damir Iveković², Fabio Faraguna³, Suzana Šegota¹, Marija Čurlin⁴, Vida Strasser¹, Jasminka Kontrec¹, Damir Kralj¹, Nadica Maltar Strmečki¹, Maja Dutour Sikirić¹

¹*Ruđer Bošković Institute, Zagreb, Croatia*

²*Faculty of food technology and biotechnology, University of Zagreb, Croatia*

³*Faculty of Chemical Engineering and Technology, University of Zagreb, Zagreb, Croatia*

⁴*School of Medicine, University of Zagreb, Zagreb, Croatia*

Recently, composite materials based on calcium phosphates (CaPs) and different TiO₂ nanomaterials (TiNMs) are emerging as potential multifunctional biomaterials for hard tissue regeneration. Among different routes for their production, biomimetic synthesis attracts special attention as an environmentally friendly and low-cost option. However, for the successful biomimetic synthesis, the detailed knowledge of the influence of physico-chemical properties of different TiNMs on CaPs precipitation is needed.

In this study, the influence of TiO₂ nanoparticles (TiNPs), nanowires (TiNWs) and nanoplates (TiNPLs), as well as titanate nanotubes (TiNTs) on CaPs precipitation was systematically investigated in two types of experiments. The ability of TiNMs to induce CaPs formation was tested in corrected simulated body fluid (c-SBF), while their influence on formation kinetics and physico-chemical properties of formed CaPs was studied in the spontaneously precipitating system.

Although TiNMs were not able to induce CaP formation in the c-SBF, they exhibited uncommon dual influence on spontaneous precipitation kinetics, i.e. inhibition of initially formed amorphous calcium phosphate (ACP) transformation at lower and promotion at higher concentrations. After one hour of reaction time, calcium deficient hydroxyapatite (CaDHA) was formed. Morphology of the CaDHA depended on the type and concentration of TiNMs.

Powder X-ray diffraction, electron paramagnetic spectroscopy and thermogravimetric analysis confirmed that there was no significant difference between CaDHA formed in the presence of different

TiNMs, which indicates that CaDHA composites with different TiNMs could be prepared at similar conditions in a much shorter time compared to the usual procedure with SBF.

Obtained results confirm the importance of surface structure and charge in CaPs – TiNMs interactions. They as well contribute to a deeper insight into precipitation processes on the nanosurfaces, which enables a rational approach in the biomimetic synthesis of novel biocomposites for hard tissue regeneration.

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OUTSTANDING OSTEOINDUCTIVE PROPERTIES OF ALBO-OS ON HUMAN DENTAL STEM CELLS

Dijana Trišić

Department of human genetics, Faculty of Dental Medicine, University of Belgrade, Belgrade, Serbia

Novel bone substitute named ALBO-OS, based on hydroxyapatite porous scaffold covered with thin polymeric film of poly(lactide-co-glycolide) (PLGA), has been synthesized recently. The aim of the study was to investigate osteoinductive potential of ALBO-OS, *in vitro*, on stem cells from apical papilla (*SCAPs*).

SCAPs were characterized by flow cytometry and multilinear differentiation. Proliferation, migration and mitochondrial activity were analyzed in presence of material. Further, ALBO-OS discs were placed in well plates and *SCAPs* were seeded on to scaffolds and in control wells. Cells were cultured in growth and osteogenic medium, and cells cultured in control medium served as control. Alkaline phosphatase activity, gene expression analysis, and qualitative Alizarin Red S staining were analyzed. The relative target gene expression of odontogenic marker matrilin-4 (*MTNR-4*), and osteogenic markers osteocalcin (*OCN*), and bone morphogenetic protein 2 (*BMP-2*) were determined by real-time reverse-transcription polymerase chain reaction.

The 98 – 99% of cells were positive for mesenchymal stem cells markers and successfully differentiated into osteogenic, adipogenic and chondrogenic lineage. Viability, mitochondrial activity and migration in presence of material and its products showed excellent biocompatibility. The relative expression of selected markers of osteogenic differentiation (*OCN*, *BMP-2*) showed to be upregulated in the presence of ALBO-OS, both in cells cultivated in growth ($p < 0.01$) and in osteogenic medium ($p < 0.01$, $p < 0.0001$). Upregulation of *MTNR-4* was noticed only in group *SCAPs* cultured in osteogenic medium alone ($p < 0.0001$), while presence of ALBO-OS influenced downregulation of odontogenic marker, in both mediums. Alkaline phosphatase was highly expressed in cells seeded on scaffold as in cells cultured in induction medium, ($p > 0.05$). Mineralization globules were observed on SEM micrographs as well as after qualitative staining.

Results of this study revealed excellent osteoinductive properties of ALBO-OS alone, while by adding induction medium osteogenic properties were further induced. Upregulation of *MTNR-4* only of *SCAPs* cultured in osteogenic medium alone could suggest that, since their dental tissue origin, *SCAPs*

spontaneously different to odonto/osteogenic lineage, but in the presence of scaffold pure osteogenic differentiation occurs.

Hazardous waste incineration plant

Nebojsa Soldatovic

Organic polluted materials (lacquer/paint sludges, solvent mixtures, industrial waste water sludges, filter cakes, pesticides, contaminated soil, absorbents, laboratory chemicals, medical waste, etc.) under thermal treatment can release significant amount of usable energy. Based on this principle, using waste incinerator, win-win situation could be achieved as the organic contaminated waste is destroyed and the energy is produced simultaneously.

The whole procedure consists of transition of liquid, solid and pasty substances through various feed paths in firebrick coated rotary kiln. During the process, they burn at approximately 1200 °C. Residues and incombustible materials (like metal) are further cooled, separated and sent to slug sorter. Furthermore, the subsequent afterburning chamber is employed to completely burn out the flue gases at approximately 1050°C. The exhaust gases go further through heat recovery boiler where they during deprivation, emit energy which can be converted into process steam or electricity. Electrostatically precipitated dusty pollutants are then stored safely in underground storage. The gases go further through a three-stage wet exhaust gas purification where they are cleaned of acid fumes, heavy metals and sulfuric dioxide. Gases are then treated with special mixture (lime-carbon mixture) which binds the remain pollutants. The next step is transmission through a fiber filter which extract pollutants from the pure gas. Purified gas is released in atmosphere while used lime-carbon mixture can be reused passing the whole process starting from rotary kiln again.