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Different conjugation strategies of gold nanoparticles with anti-SARS-CoV-2 antibodies for ultrasensitive detection of coronavirus

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In recent years, gold nanoparticles (AuNPs) have attracted vast interest in biosensing applications. Their unique functional and surface plasmon resonance properties, coupled with possibility to bound various biomolecules (e.g., antibodies, DNA, and RNA aptamers) may significantly enhance diagnostic features. Therefore, functionalizing AuNPs with antibodies could be a new approach for detecting COVID-19 caused by SARS-CoV-2 virus (1, 2). Here we present three conjugation strategies in order to assure efficient binding of monoclonal anti-SARS-CoV-2 antibodies to AuNPs in the oriented way: a) direct conjugation of antibodies by electrostatic interactions or physical adsorption on AuNPs, b) conjugation mediated by glycopeptide-functionalized AuNPs using peptidoglycan monomer, GlcNAc-MurNAc-L-Ala-D-*iso*Gln-*mes*oDAP(NH2)-D-Ala-D-Ala (PGM)) and c) covalent conjugation using EDC/NHS method. The AuNPs-antibodies conjugates were characterized by various spectroscopic and electrophoretic techniques. Additionally, gold nanoparticles labelled with secondary antibodies (Immunogold) were used to determine the amount of available epitopes of the primary monoclonal antibodies on the metal surface. Preliminary results showed that binding efficiency of antibody-AuNPs conjugate can be improved using the covalent binding strategy with their high potential for use in SARS-CoV-2 virus detection.

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Artificial neural network and support vector machine regression for forensic age determination using Raman spectra of teeth

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Age determination is a common procedure in investigating human remains. There are different age determination techniques available today, however, they are either subjective and based on the investigator's experience, not accurate enough, or expensive and time-consuming. Teeth are the most lasting tissue of the human body and are usually found undamaged. Raman spectrometry of teeth offers non-destructive analysis without any previous sample preparation and has clear advantages for forensic purposes. For this study, a sample of 71 teeth was used from donors aged between 11 and 76 years. Raman spectra were recorded using FT-Raman accessory with a 1064 nm laser on three distinct spots on the tooth, crown, neck and apex. Spectra were analyzed using principal component regression (PCR) (1), support vector machine (SVM), and artificial neural network (ANN). This study showed that age determination can be achieved using regression techniques and that the result of classification models depends on both, the recording site on the tooth and the technique used. Models built with ANN and SVM, especially when built with spectra recorded on the apex, showed better results than those obtained using PCR. The coefficient of correlation, R^2, varies with the number of PCs used, which suggests that optimizing the selection of PCs used might improve the final classification accuracy of the model. Difference between male and female teeth occurs regardless of the technique used.

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Cellular model of Parkinson's disease for safety testing of gold-based nanodelivery system

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Parkinson`s disease is the second most common neurodegenerative disorder (1). It is characterized by early prominent death of dopaminergic neurons in substantia nigra which results in classical motor symptoms of the disease: rigidity, rest tremor, bradykinesia, and postural instability (2). Although initially described more than 200 years ago (3), not a single drug is able to alter the natural history of the disease. Mainstay therapy is levodopa - a precursor in the synthesis of the dopamine - that can cross the blood brain barrier. Since introduction to the therapy, 50 years ago, many improvements have been made to enhance its efficiency and reduce side effects. However, passage of the blood brain barrier is still relatively poor, side effects can pose significant burden and long-term therapy is associated with motor complications. Motor complications are, at least in part, due to the fluctuations of levodopa concentrations in the plasma. In many cases these complications can be so intense that the only options is to discontinue the drug which inevitably leads to rapid decline in the quality of the life. Therefore, new formulations of levodopa, that can increase delivery of the drug to the brain and reduce plasma fluctuations are needed. This study tested the safety of phosphoglucomutase (PGM) functionalized gold nanoparticles (AuNP) as levodopa carrier using differentiated neuroblastoma (SH-SY5Y) cells as *in vitro* model of Parkinson`s disease. AuNPs shape, size, and surface charge were determined with transmission electron microscopy (TEM), dynamic light scattering (DLS), and zeta potential measurements, respectively. Cell viability and apoptosis induction test were performed using flow cytometry, while oxidative stress was determined by measuring the concentration of reactive oxygen species, glutathione (GSH) and mitochondrial membrane potential. In all experiments levodopa loaded AuNPs were compared with AuNPs and levodopa alone.

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Azide-alkyne click reactions in vapor phase processes

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Vapor phase processes have been developed for building organic-inorganic hybrid films using small molecules from the gas phase on almost all kind of surfaces. The advantages of these processes include automatization, scalability, uniform coating even of porous substrates and excellent film thickness control at the nanoscale, which make them very interesting for applications, ranging from electronics to biomedical devices. The most known, atomic layer deposition (ALD) is used for the growth of inorganic thin films from organometallic precursors. Similarly, molecular layer deposition (MLD) is used to grow organic polymeric thin films by employing gas organic monomers as precursors, and further combinations of ALD/MLD can afford modular hybrid structures. Although several MLD processes are known today, there is still plenty room for investigation given the vast possibilities provided by the organic chemical reactions. However, the choice of organic precursors is challenging because several factors (e.g., volatility, stability, reactivity) must be considered to expand the scope of the method. Our efforts toward the introduction of azide-alkyne click chemistry in ALD/MLD processes will be presented. This concept has been demonstrated in pulsed vapor phase surface functionalization of metal oxide surfaces (1, 2).

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Multilayers of polyaminoacids and silver nanoparticles as antimicrobial coatings for orthopaedic implants

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Orthopaedic implant failure is a major health problem. The main cause is nosocomial infections, which are further complicated by the increased incidence of antimicrobial resistance (AMR). This fact motivates the research of alternative approaches in the development of antimicrobial materials and, in particular, new surface coatings that can locally prevent microbial adhesion and proliferation (1). Recently, multilayers of biocompatible polyelectrolytes containing antimicrobial components have been investigated because of their wide application on different types of implants. In this study, a multilayer of poly-L-glutamic acid (PGA) and poly-L-lysine (PLL) with incorporated silver nanoparticles (AgNPs) was formulated and physiochemically characterised. AgNPs were selected because they have antimicrobial properties and do not promote the development of AMR (2). AgNPs were synthesised by reduction of silver nitrate with polyvinylpyrrolidone as stabilising agent (3). The synthesised nanoparticles were characterised by transmission electron microscopy (TEM) and UV-Vis spectroscopy. Their stability in ultrapure water and buffer (HEPES/NaCl) with the addition of PGA or PLL was determined by monitoring the hydrodynamic diameter and zeta potential during 24 hours. The most stable suspension was used for the layer-by-layer assembly of polyaminoacids multilayer with or without incorporated AgNPs, structured as follows: $(PLL\text{-}PGA)_{10}$ and $[(PLL\text{-}PGA)_2\ (PLL\text{-}AgNP)\ (PLL\text{-}PGA)_2]_2$ respectively. The assembly was monitored by quartz-crystal microbalance with dissipation monitoring (QCM-D) and atomic force microscopy (AFM). The QCM-D measurements showed that the adsorption of the consecutive individual layers of polyaminoacids and nanoparticles was accompanied by a stepwise decrease in frequency, indicating successful assembly of the multilayer. Both in the presence and absence of AgNPs, the exponential growth regime was observed. The increase in dissipation indicated the viscoelastic properties of the both type multilayers. AFM micrographs after five adsorbed bilayers showed granular structures that became larger and denser after adsorption of additional layers. The difference in the morphology of the multilayers with and without incorporated AgNPs indicates their successful incorporation. In summary, the results indicate that AgNPs can be successfully incorporated into polyaminoacids' multilayers, which is of potential interest not only for the development of new coatings for orthopaedic implants but also for other biomedical materials.

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HOuDiNI – synthesis of Ceria nanocatalyst

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In this short presentation, the work of the research group of prof. Kurajica operating at the Department of Inorganic Chemical Technology and Non-Metals at the Faculty of Chemical Engineering and Technology is demonstrated. The research group deals with advanced methods of preparation of inorganic nanomaterials, such as hydrothermal and sol-gel methods, mechanochemical synthesis and combustion synthesis. Regarding the type of material, the emphasis is on metal oxides (CeO_2, Al_2O_3, TiO_2, ZnO, VO_2, etc.) and perovskite materials ($CaTiO_3$, $AlCeO_3$) with primary applications in catalysis and photocatalysis, but other applications such as sensors, smart materials and UV filters have been studied. Methods of nanomaterials characterization directly accessible and proficiently used by the group members include X-ray diffraction analysis, thermal analysis techniques (differential thermal analysis, differential scanning calorimetry, thermogravimetric analysis), UV-Vis diffuse reflectance spectroscopy, Fourier-transformed infrared spectroscopy, energy dispersive X-ray spectroscopy, scanning electron microscopy and atomic force microscopy. Croatian Science Foundation project on which the research group has worked intensively for the past 4 years called *Hydrothermal synthesis of doped ceria nanocatalyst* has resulted in 21 papers in journals, of which 17 in CC journals, 2 papers in conference proceedings, 21 conference abstracts, several Bachelor's and Master's theses, and 1 Rector's Award so far. The project is coming to an end soon and the members of the group are interested in achieving new collaborations and new projects.

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Optimizing bilayer organic *pn* junctions for photo-capacitive stimulation

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Thermally evaporated thin films of donor and acceptor type organic pigments on conductive back electrodes make organic photo-capacitive devices that can be used to wirelessly stimulate nerves (1) and cells (2) when illuminated with light. We focus on devices made from metal-free phtylocyanine (H2PC) and N,N'-dimethyl perylenetetracarboxylic diimide (PTCDI) as acceptor and donor type molecules respectively and ITO or thin gold film as the back electrode. Such devices are biocompatible, fully wireless and stable in an aqueous environment. Measuring and numerically simulating electrical potential distribution created by this type of device in response to light illumination enables us to design devices in a way to have stronger and more focused electric fields that enable localized, target specific, stimulation. We do this by optimizing the fabrication process, precisely controlling the thicknesses of the films and by 3D structuring the device geometry (3).

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Biocompatibility assessment of selenium nanoparticles as potential drug delivery system

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Parkinson's disease (PD) is neurological disorder characterized by the loss of dopaminergic neurons. Because of that, dopamine levels are reduced and main consequences are bradykinesia, rigidity, tremor and depression (1). Levodopa is the most common therapy for PD but long-term treatment can have a lot of side effects. In order to that, drug delivery system needs to be designed for improving targeting ability. Selenium nanoparticles (SeNPs) constitute an attractive carrier platform because of their antioxidant properties and neuroprotective effects (2). This study aimed to develop drug delivery system consisted of selenium nanoparticles coated with polyvinylpyrrolidone (PVP) and polysorbate 20 (Tween 20). Differently coated SeNPs were prepared via reduction of sodium selenite by L-ascorbic acid. Synthesized nanoparticles were characterized with dynamic light scattering (DLS) and transmission electron microscopy (TEM) for determining their shape and size. *In vitro* model of blood-brain barrier (BBB), hBEC-5i cell line, was used in all experiments. Cell viability testing, oxidative stress response and permeability evaluation of BBB for SeNPs were used in biocompatibility assessment. For oxidative stress response experiments 3 different assays were used: dichlorodihydrofluorescein diacetate (DCFH-DA), dihydroethidium (DHE) and rhodamine 123 (Rh123). In transwell experiments cells were treated with SeNPs alone and in combination with L-dopa. Concentration of Se in all compartments (donor well, acceptor well, cells, gelatin) was analyzed by Graphite Furnace Atomic Absorption Spectroscopy (GFAAS) while L-dopa concentration was determined by commercial ELISA kit. Results showed that SeNPs in concentration of 1 mg Se/L did not reduce the cell viability significantly and that SeTween NPs have lower level of ROS production than SePVP. Transwell experiments resulted in low permeability for both SeNPs type but *in vitro* assays that were used in this study were not suitable for evaluating the BBB permeability of SeNPs because of their sticky properties.

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Whispering gallery mode spherical microspheres; sensing and nonlinear optical application

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Microsphere resonators have increasingly attracted interest because they have the ability to become keystone in photonic circuit devices, optical communications, sensing, and lasing applications. As one of the essential categories of optical microcavities, microsphere resonators based on whispering gallery modes (WGMs) have benefits of ultra-high Q factor, small mode volume, ease of fabrication, and low cost. They have been extensively studied for nonlinear optical applications. As yet, the majority of microsphere resonators are made of SiO_2. In recent years chalcogenide glass spherical microspheres acquired considerable attention due to their unique properties. Our work is mainly focused on WGM microspheres sensing and nonlinear optical applications. We studied the refractometric gas sensing sensitivity and the effect of thickness of the sol-gel silica layer coated to the microsphere to detect small traces of ammonia. Silica microspheres were produced by melting the tip of a standard silica fiber using an electric arc from a commercial fiber splicer. A porous silica layer is made using the sol-gel method and it is coated to the sphere using dip coating. The microspheres made using this method have high Q modes in the range of 10^5 to 10^6. For ammonia gas sensing measurements, a signal from a tunable laser is coupled to the coated microsphere using a tapered optical fiber. Both the sphere and the taper are placed in an isolated testing chamber. The desired concentration of ammonia in the chamber was controlled by mixing 100 ppm NH_3/Ar mixture with Ar using two mass flow controllers. The study reveals that different modes of the same microspheres have different sensitivities. It is concluded that the observed whispering gallery modes have different values of the radial index p. To acquire high sensitivity, it should be selectively coupled to p=0 modes. In addition to the sensing application, microspheres made up of chalcogenide fibers (As_2S_3, As_2Se_3) were reported recently. Our aim is to fabricate microspheres using different chalcogenide materials and to carry out a comparative study of nonlinearities. Microspheres are coupled using a tapered silica fiber and we fabricated chalcogenide microsphere of quality factor 10^7. Furthermore, Microspheres are suitable to generate four wave mixing process and frequency combs. In the past few years, WGM microspheres research is thriving because of their tremendous applications in optical communications and material processing. Efforts are underway to fabricate high quality chalcogenide microspheres and to study the nonlinear properties.

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Identification and quantification of microplastics in bottled water by micro-raman spectroscopy

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Microplastics (MPs) are defined as small plastic particles in micro scale, typical range is between 1 μm and 5 mm. Even though there is still not defined size range of microplastic, plastic particles below 1 μm are referred to as nanoplastics. MPs enter the environment uncontrollably either by macroplastic decomposition or as particles deliberately produced in micro size. Although there is not yet much findings about microplastics and its impact on human health, limited number of research have stated that every adult person and child might ingest from dozens to 100 000 particles everyday. Up to now, only a few studies about microplastic contamination of bottled mineral water have been published. Therefore, we are focused on development and optimization of method for identification and quantification of MPs by micro-Raman spectroscopy. Bottled water is investigated for microplastic contamination by filtering water on silicon filters which are then analyzed by micro-Raman spectroscopy down to 1 μm. Both still and sparkling water are the subject of research. As we have just started the research, the obtained results are the first preliminary results which are an indicator of whether there is contamination of Croatian bottled water with microplastics. We have detected microplastic in bottled still water and sparkling water, mainly PE, PET and PP.

Investigation into the physico-chemical properties of bacterial nanocellulose membranes after an alkali purification process

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Bacterial nanocellulose (BNC) is an extracellular, exceptionally versatile natural biomaterial produced by some nonpathogenic bacterial strains. It is a chemically purest cellulose with a unique nanofibrillar structure (1, 2). Unlike plant cellulose, BNC is free of hemicellulose, pectins, waxes, lignin, or other lignocellulosic components. Despite its high purity, it contains some impurities in the form of cell biomass and/or medium components, which can be easily removed using alkali treatment (sodium or potassium hydroxide), organic acid treatment (acetic acid), repeated washing with distilled water or hot tap water, or a combination thereof (3). But the choice of purification method can damage the structure of the nanocellulose and affect its physico-chemical properties. The aim of our work was to determine the effect of the NaOH purification process on the properties of the BNC membranes. 13 strains of acetic acid bacteria were tested for the production of BNC membranes. Among them, a novel bacterial strain *K. melomenusus* AV436^T was chosen for the production of BNC. The BNC membranes were produced by aerobic acetic acid bacteria under static fermentation conditions. The bacterial culture was incubated at 30 °C for 3 days in 50 mL of RAE medium. The membranes were produced on the air-liquid interface and purified by 0.5% w/v NaOH solution at different immersion times (15 min, 30 min, 1 h, and 2 h) and constant temperature of 80 °C. Further treatment included washing in ultrapure water till neutral pH and drying at 50 °C for 24 h. The effect of an alkali purification process on physico-chemical properties of BNC membranes was evaluated using different characterization techniques, including mass determination using gravimetry, wettability through contact angle goniometry, morphological structure changes using SEM, chemical structure analysis through FTIR, and crystallinity determination using XRD.

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