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INVITED SPEAKERS

Id-334

Thin Films of Oxides Grown by ALD - New Bio and Medical Applications

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Abstract: Technology of Atomic Layer Deposition (ALD) was applied for deposition of thin films of selected wide band gap oxides on different substrates, including the temperature-sensitive one. Thin films deposited by the ALD show several encouraging properties. The films are very dense, are pin holes free, and the ALD method allows control of their electrical and optical parameters. Despite of slow growth rates, several practical applications of the ALD-grown films are demonstrated. For example, ALD-grown ZnO and doped ZnO films are intensively studied for applications in photovoltaics, as transparent electrodes, in transparent electronics, and in optics. For the latter application nanolaminar structures of oxides deposited by the ALD are very promising materials. Other applications include dielectrics such as Al2O3, HfO2, ZrO2, TiO2 as anti-reflection layers, passivation layers of back contacts in silicon-based solar cells, and socalled gate oxides. Some examples of such applications will first be given. Surprisingly, our recent investigations suggest a new extremely important areas of applications of the same (as mentioned above) wide band gap oxides. These oxides show effective anti-bacterial (antimicrobal) activity against so-called "hospital bacterial strains". Their use will allow to replace widely used (in fact too widely) antibiotics. Importantly of this new application, oxide films can be deposited at low temperature, allowing coating of range of materials used in clinics, including temperature sensitive ones. A new application (coating of implants) will be also demonstrated. Acknowledgments: The present research was partly supported by the NCBR National Centre for Research and Development) project TECHMATSTRATEG1/347012/NCBR/2017.

Keywords: Oxides, Thin films, Bio Applications.

INVITED SPEAKERS

Id-374

Matrices of Gas Aggregated Metal Nanoparticles for Enhancement of SALDI MS

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Abstract: Matrices of nanoparticles (NPs) deposited on surfaces are often used for the enhancement of sensing and detection. One of the examples is surface enhanced Raman spectroscopy (SERS), where strong local electric fields generated by metal NPs exhibiting localized surface plasmon resonance (LSPR) on interaction with laser light leads to a tremendous increase of detection efficiency. Another field of application is matrix assisted laser desorption ionization (MALDI) mass spectrometry (MS). When NPs deposited on the surface are utilized as matrices, the technique is called surface-assisted LDI, i.e. SALDI. This method is very widely used for the analysis of biomaterials because of its ability to provide desorption and ionization of high-mass thermally labile molecules. However, the mechanisms of detection enhancement provided by NPs are poorly studied, in particular, how heat is absorbed by NPs and then transferred to the analyte originating desorption, how the ionization undergoes and what is the role of metal ions. It is argued in recent publications that LSPR can play an important role in thermal ionization. In this work, NPs of copper and silver were produced by gas phase aggregation method in vacuum magnetron sputtering cluster apparatus called MaSCA. Advantages of the setup are in the formation of very pure monocrystalline NPs, the ability of size selection and good control of NP surface coverage. The deposited NPs were studied by atomic force microscopy to evaluate particle sizes and surface coverage as well as to investigate the effects of laser irradiation on the NP shapes and distribution. Matrices of metal NPs were tested in the detection of riboflavin molecules as well as of phospholipids, fatty acids and other species composing pig brain and beeswax homogenates. 3rd harmonic of Nd:YAG laser at 355 nm and mass spectrometers from ThermoFisher Scientific and Bruker Daltonics were utilized. Nano-PALDI MS measurements were carried out for different particle sizes and laser energies as well as for both positively and negatively charged ions. The studies revealed higher enhancement of mass spectra for the case of silver compared to copper which is argued to be

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related to the laser wavelength. It is closed to the LSPR wavelength of silver NPs, thus, the plasmonic absorption can significantly facilitate heating and ionization of the analytes. The study also shows clear dependences of detection efficiency on particle size and laser energy. The latter significantly affects the NPs: they become molten and partly sublime/evaporate at high laser fluxes.

Keywords: Matrices of Metal Nanoparticles, Surface-Assisted Laser Desorption İonization.

INVITED SPEAKERS

Id-377

Bimetallic Based Nanostructures for Electrochemical Sensing Applications

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Abstract: Innovative techniques and strategies for the production of nanomaterials and nanostructures have advanced as promising tool to develop a new class of modified electrodes applicable in electrochemical biosensors. The combination of nanotechnology with modern electrochemical techniques has paved the way for developing different bimetallic nanoparticlesbased sensors very efficient for clinical, food and environmental analysis. Bimetallic nanoparticles due to the synergistic effect are found to be multifunctional and act as high-energy surface sites enhancing the electron transfer kinetics between electrode-electrolyte interfaces. Also, the bimetallic nanoparticles having both accurately and precisely controlled structures and compositions are highly advantageous due to their strong relationship with properties. The finetuning of the bimetallic nanoparticle improves the electrocatalytic ability which directly influences the sensing abilities. Therefore, the various strategies for enhancing the catalytic activity of bimetallic nanoparticle surfaces such as strain, ligand, and ensemble effect can be taken into consideration during the preparation of bimetallic nanoparticles for ameliorating their sensing abilities. Electrochemical techniques are particularly attractive for the electrodeposition of (bi)metallic nanostructures on the surface of the electrode not for mass preparation in the bulk solution. Recent approaches we have used in the development of bimetallic nanostructures for modified electrode architectures will be presented, illustrated with applications to the sensing of analytes important for clinical and environmental samples (glucose, dopamine, nitrite). These have included the electrosynthesis of Ni-Co nanoparticles with different sizes and distributions comparatively electrodeposited on three carbon materials (graphene, carbon nanotubes and fullerene) and assessed towards oxidation of glucose. Likewise, bimetallic Ag-Au electrodeposited using a double pulsed technique enable us to control the silver and gold content in the electrodeposited nanoparticles and to optimize the response to dopamine. Also, codeposition of AuNPs together with MoS2 has proved to displayed excellent properties for nitrite electrochemical oxidization. This work was supported by a grant of Ministry of Research

and Innovation, CNCS - UEFISCDI, project number PN-III-P4-ID-PCCF-2016-0050, within PNCDI III.

Keywords: bimetallic nanoparticles, electrodeposition, electrochemical sensors

INVITED SPEAKERS

Id-382

Chemical Nano Biosensors Based on Novel Phenomena in Langmuir and Langmuir-Blodgett Films from a Lipids and Phospholipids

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Abstract: Environmental monitoring of novel important pollutants in air and water currently is performed on expensive instruments in a laboratory. The possibility to measure in the field and in real-time is almost non-existent. Biosensors are recognized by both the scientific and industrial communities as the most promising alternative to fill this gap. For the preparation of the sensing layer in a chemical sensor, the Langmuir-Blodgett (LB) method is considered the best alternative for supramolecular architecture. An insoluble monolayer from organic molecules with suitable biphylic balance is formed and investigated at the air-water interface (Langmuir film). Subsequently, a transfer layer by layer on a substrate with controlled molecular orientation, density, and phase is performed. In the past 30 years we have systematically investigated fluorescently labeled phospholipids, mainly DPPE head labeled with NitroBenzoxaDiazole (DPPE-NBD). Mimicking the molecules in biological membranes, these molecules are a suitable matrix for insertion of selectively reacting proteins, enzymes, aptamers in the sensing layer of a biosensor. LB layers from only phospholipids and lipids show promising gas sensitivity to volatile organic compounds (VOCs) with very fast reacting times and complete reversibility on gas removal. Three new effects were discovered by us in Langmuir and LB films from DPPE-NBD which further enhance their use in nano biosensor applications. Fluorescence self-quenching was observed when molecules are in a condensed phase close to each other. Insertion of large bivalent ions in the water subphase increases the distance between the molecules and thus the fluorescence signal was recovered. This effect was used for the detection of the very harmful Cadmium ions in water at very low concentrations. Formation of 3D needle-like structures at thermodynamic equilibrium for single component monolayers was first observed for this molecule. This new effect is especially important for biosensor applications because the surface of the sensing layer is significantly increased while keeping the volume to a minimum. Thus, fast

and very sensitive sensors are possible and this is an alternative method of increasing the surface-to-volume ratio in sensing layers. Transduction of signal from the sensing layer to a measurable output in our research was performed with all 3 main methods: optically by measuring fluorescence intensity and kinetics; electrically by electrical impedance spectroscopy; and gravimetrically by using a two-port Reilegh type Surface Acoustic Wave resonant devices with a resonant maximum around 430 MHz. These SAW resonators exhibit 4000 times higher sensitivity compared to standard 10 MHz QCM devices. This work was supported by contract numbers: KP-06-OPR 03/9 and KP-06-Russia/8 with the Bulgarian National Science Foundation.

Keywords: Langmuir Films, Langmuir-Blodgett Films, Nano Thin Films, Chemical Biosensors.

INVITED SPEAKERS

Id-388

Antibacterial Properties of Si₃N₄ Based Ceramics

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Abstract: Over the past two decades, a significant amount of interest has been generated for the bio-applications of silicon nitride-based ceramics especially in orthopedic applications because of their significant advantages of the combination of mechanical properties and biologically acceptable chemistry. However, biological applications require additional properties, enabling stronger chemical bonding to the surrounding tissue for better fixation and the prevention of bacteria biofilm formation. Infection caused by the bacteria is a major problem in any kind of material leading to failure and it is a well-known fact that due to the failure in treating infection, revision surgeries have become far too common for orthopedic devices. Even though Si3N4 is known to be more favorable among some other biomaterials (like Ti and PEEK), the studies based on hindering infection is still quite important to increase the success in their orthopedic and/or dental applications. Therefore, in this study, different factors affecting bacterial response such as different sintering additives and surface chemistry were investigated. Surface chemistry examination revealed a reduction in grain boundary phase amount in SiAION ceramics compared to Si3N4, and Si3N4 with higher grain boundary phase amount showed a more hydrophilic character. The antibacterial behavior against E. coli was similar between the two systems but an improvement was observed for Si3N4 due to the combination of the hydrophilicity of Si3N4 and a reduction of grain boundary phase amount. Effect of sintering additives was investigated in glass systems and the results revealed that the antibacterial behavior and mammalian cell viability were inversely correlated in glasses with different cation additions. Ca was the best cation when the two properties (bacterial response and cell proliferation) were considered together, and, thus should be further studied for a wide range of applications.

Keywords: Silicon nitride, SiAION, Bioceramic, Antibacterial

INVITED SPEAKERS

Id-391

Electrochemical Biotransducers for Label-Free Analysis of Biomolecules: from Proof of Concept to Medical Applications

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Abstract: In the third generation of biosensors, the action of the biomolecular recognition component is directly interfaced with the physico-chemical transducer, generating a good reading of a biological recognition reaction. Because biomolecular analysis is very important in various fields of application (clinical diagnosis, food analysis, environmental monitoring, pharmaceutical development). The use of enzymes, as components of biomolecular recognition, offers great advantages for real-time analysis. Enzymes are widely used in conjunction with electrochemical transducers, due to its high specificity and sensitivity. The presentation will highlight biosensors development in our laboratory from simple to complex architecture. A layer-by-layer self-assembled biosensor for glucose with a low operation potential of -0.2 V vs. Ag/AgCl achieved a detection limit of 41µM was developed by glucose oxidase-based biosensor. Acetylcholinesterase was immobilized using sol-gel methods to develop an inhibition based biosensor for heavy metal ion monitoring, showing promising results (LoD = 0.19 μ g/L for Cd²⁺ ions) to lay the background for portable instrumentation for environmental monitoring. A tyrosinase-based biosensor was developed for the selective detection of dopamine with an LoD of 0.43 µM in the presence of dopamine medication, with satisfactory results in terms of recovery (96%), and relative standard deviation values below 5%. Last, biosensor architectures based on the enzyme-like catalytic activity of gold nanoparticles will be presented. The before mentioned nanozymes address the limitations of natural enzymes and conventional artificial enzymes and allow modulation of activity and selectivity through green synthesis pathways. Thus, gold nanoparticles were successfully used for the detection of hydrogen peroxide by means of its quenching mechanism in the presence of antioxidant compounds. Green synthesized gold nanoparticles were incorporated in phospholipid films for highly selective and sensitive oxidative stress biomarker detection (H₂O₂). These results confirmed the applicability of the biosensors in real samples such as human urine and blood serum, towards achieving point-of-care diagnostics. This work was supported by a grant of the

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Keywords: Biotransducers, Electrochemical, Enzymes, Nanozymes.

INVITED SPEAKERS

Id-400

Self-assembly of Amphiphilic Triblock Copolymers into Versatile Sensing Platforms Using a Microfluidic Approach

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Abstract: Due to their ability to form biomimetic membranes, amphiphilic block copolymers have recently emerged as attractive alternatives to biological amphiphilic species (lipids). Besides resembling the structure of biomembranes based on phospholipid counterparts, polymer membranes are endowed with chemical versatility, a much higher mechanical stability and improved robustness. Amphiphilic triblock copolymers (TBCs) are excellent candidates for mimicking biological membranes due to their specific chemical structure consisting of two outer hydrophilic blocks and a central hydrophobic block. While TBCs of ABA type consist of two identical hydrophilic parts (A) and a longer hydrophobic block (B) that self-assemble into a symmetric membrane, the asymmetric structure of ABC-type TBCs allows for the formation of asymmetric, oriented membranes. Here, the two chemically different water-soluble blocks A and C mimic the hydrophilic heads of lipids forming a bilayer membrane, whereas the hydrophobic part B of the copolymer resembles the domain formed by interdigitation of hydrophobic tails of lipids. Both ABA and ABC TBCs can self-assemble into symmetric (ABA) or asymmetric membranes (ABC) that can serve as a platform for directed insertion of biomolecules. To enhance the preferred orientation and distribution of inserted biomolecules, the membrane thickness and its functionalization can be further tailored by careful selection of polymer blocks and their length, as well as the choice of self-assembly method. In this work, we explore the formation of polymer membranes via a microfluidic approach by self-assembly of both kinds of TBCs onto a porous solid support previously immersed in an aqueous solution containing an enzyme. After the formation of a membrane the separation of two chemically different aqueous solutions using the assembled TBC-based membrane occurred. Following, the insertion of a pore protein allowed us to engineer a fluorescent biosensor platform, where the enzyme trapped in the pores could sense the substrate molecule from the outer solution that diffused through the membrane. While the systems obtained here have been evaluated using a model based on horseradish peroxidase for chemical detection of hydrogen peroxide, used for indirect sensing

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of glucose in biological fluids, these sensing platforms can be easily tailored and adapted for targeted sensing applications by adequate selection of an enzyme and substrate combination. **Keywords:** Self-Assembly, Triblock Copolymers, Biomimetic Membrane, Transmembrane Sensing, Biomolecule İnsertion.

REGULAR SESSIONS

Id-327

Ectopic Ostegenesis of Bioinspired Composite Scaffold with Graphene Oxide Filling and Hydroxyapatite Gradient Density

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Abstract: Herein, the synthesis and characterization of novel chitosan-gelatin highly porous scaffold reinforced with graphene oxide and hydroxyapatite (HAp), crosslinked with genipin was targeted. In tissue engineering, chitosan and gelatin are two of the most robust biopolymers with wide applicability, due to intrinsic biocompatibility, biodegradability, low antigenicity properties, affordability and ease of processing. HAp, per its exceptional activity in tuning cell-matrix interactions, is acknowledged for its capability of sustaining cellular proliferation by promoting bone-like native micro-media for cell adjustment. Genipin is regarded as a top class cross-linker, while graphene oxide (GO) is viewed as one of the most performant and versatile fillers. The composites with natural bone HAp/biopolymer ratio were obtained by cascading sonochemical treatments, followed by uncomplicated casting methods and by freeze-drying. Their structure was characterized by Fourier Transform Infrared Spectroscopy and X-ray Diffraction, while overall morphology was investigated by Scanning Electron Microscopy (SEM) and micro-Computer Tomography (µ-CT). Ensuing that, in vitro enzyme degradation was performed to detect the most promising compositions for the development of in vivo assays. Suitable GO dispersion was ascertained within the biopolymer mix as nanolayers specific signals lack in both FTIR and XRD spectra and the specific spectral features of the polymers persisted with GO load enhancement. Overall, correlations between the GO induced material structuration, crystallinity variations and chemical interaction of the compounds can be correlated with the physical features and bioactivity of each composite formulation. HAp presence reflected in SEM and µ-CT onto the pore smoothness, a requisite for cell attachment mainly highlighted within ex-vivo specimens. Moreover, the HAp distribution within follows an auspicious density gradient tuned for hybrid osseous/cartilage matter architectures which was mirrored in the mice model tests. Hence, the synthesis route of a natural polymer blend/hydroxyapatite-graphene oxide composite material is anticipated to emerge as influential formulation in bone tissue engineering.

Keywords: Composite materials, Polymer blend, Graphene oxide, Natural polymers, Hydroxyapatite.

REGULAR SESSIONS

Id-368

Portable Surface Plasmon Resonance Detector for COVID-19

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Abstract: Currently, COVID-19 diagnostics is most often based on nucleic acid detection (PCR) methods. These methods, although generally considered effective, are characterized by quite a long time-to-result and the necessity to prepare the material taken from the examined person -RNA isolation. For this reason, new detection methods are being sought, especially those characterized by high speed of the whole analysis process from the moment of sampling to the result. Currently, serological methods of detecting antibodies against the virus in the patient's blood plasma attract a lot of attention. Such methods, although less precise in terms of determining the current infection, allow to shorten the time of analysis to even several minutes, which makes it possible to consider them as a promising method of performing screening tests in people with suspected infection. The aim of the presented work is to investigate the feasibility of a surface plasmon resonance (SPR)-based detection system for COVID-19 diagnostics. A simple to use portable device has been proposed for the purpose of fast detection of anti-SARS-CoV-2 antibodies in human plasma. The device utilizes surface plasmon resonance phenomenon and is based on single-use removable cartridges. First the most advantageous peptides derived from SARS-CoV-2 virus that can serve as probes for antibody detection in patient plasma were investigated. Then the process of antibody detection using the peptides has been examined under laboratory conditions on a commercially available SPR device. The portable device has been prepared and tested on samples from humans. The detection results have been compared with results obtained in the same patients by standard diagnostic methods. This research was funded by IDUB against COVID-19 project granted by Warsaw University of

Technology under the program Excellence Initiative: Research University (IDUB). Grant title:

Rapid COVID-19 diagnostics with the use of surface plasmon resonance sensors.

Keywords: Surface Plasmon Resonance, Portable Devices, Label-Free Detection.

REGULAR SESSIONS

Id-376

Evaluation of Antioxidant Properties Using Electrochemistry Combined with In-vitro Peroxidation and Reducing Assays

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Abstract: Antioxidants are defined as compounds that can delay, inhibit, or prevent the oxidation of oxidizable materials by scavenging free radicals and diminishing oxidative stress. Most of the natural antioxidants of plant origin belong to the phenolic and polyphenolic class of compounds as well as carotenoids and vitamins. Additionally, the antioxidant potency may be related to the presence of other compounds like alkaloids. Unlike phenolic compounds, alkaloids are nitrogenous compounds of low molecular weight, mainly produced by plants for defense. Many of these alkaloids have been in use in modern medicine for various purposes. Piperine, as the most abundant alkaloid in pepper, gained a lot of attention for possible antioxidant and therapeutic properties.

In our study, electrochemical techniques were applied to widely evaluate the redox behavior of piperine by comparison to that of well-known antioxidants: ascorbic acid, protocatechuic acid, syringic acid, tyrosine and capsaicin used as controls. Electrochemistry is particularly useful to characterize the reducing potency of a compound, and controlled-potential techniques, such as cyclic voltammetry is commonly applied to rapidly assess the possible antioxidant activity. Also, electrochemistry was involved in an innovative way to investigate the potential antioxidant properties of piperine combined with different in vitro peroxidation and reducing assays: (i) 1,1-diphenyl-2-picryl-hydrazyl free radical (DPPH) scavenging; (ii) 2,2,6,6-tetramethylpiperidinyl-1-oxy (TEMPO) scavenging; (iii) ferric ions (Fe3+) reducing power; (iv) hydrogen peroxide (H2O2) scavenging. Results show that piperine readily reacts with highly oxidizing radicals and bind redox-active metal ions in a similar manner as antioxidants used as model.

As a subsequent objective of this study, we demonstrate that a combination of electrochemical method (cyclic voltammetry and differential pulse voltammetry) and electron transfer reaction-based assays could provide insightful data about the ability of antioxidants to intercept free

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radicals. The method proved to be a simple alternative to the spectroscopic based assays. This work was supported by a grant of the Romanian Ministry of Education and Research, CNCS - UEFISCDI, project number PN-III-P4-ID-PCE-2020-1523, within PNCDI III".

Keywords: Piperine, Electrooxidation, Potential Antioxidant Properties, Radical Scavenging.

REGULAR SESSIONS

Id-381

Cell-Friendly Hydrogel Fiber Fabrication for Biomedical Applications

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Abstract: Bioprinting is a powerful flexible approach with great potential for modern medicine. Despite significant progress in tissue engineering, precise hollow-core fiber structure formation technology remains a bottleneck problem. Currently, biofabrication strategies of mimicking blood vessels or bile ducts are time consuming, multistage and cumbersome. At the same time, single-step printing of implantable structures, containing living cells represents a persistent trend in modern biofabrication. Here, we report a versatile cell-friendly photopolymerization approach that enables single-step prototyping of hollow-core as well as solid-core hydrogel fiber constructs. This approach was performed by extrusion of HaCaT cell-laden hyaluronic acid glycidyl methacrylate bioink in aqueous solution containing preliminary activated initiator. We chose endogenous flavin mononucleotide with triethanolamine as a photoinitiation complex capable for excitation under cell-amiable 450 nm light irradiation. Free radical penetration from water to the structure being extruded initiated cross-linking initially occurring at the structure rim and progressing inwards. This governed a spatially-limited hydrogel gelation cross-section profile typically shaped as a concentric ring. As such, cross-linked hyaluronic acid glycidyl methacrylate hydrogel fibers present favorable microenvironment for living cells, while the fabrication technology enhances cell viability during printing. In vitro studies confirmed that single-step printed hydrogel structures are friendly to primary neuronal cultures and do not lead to significant morphological changes or loss of functional activity of neuron-glial networks. Good

biocompatibility of the constructs was shown *in vivo* indicating new prospects for their application for the central nervous system. We conclude that our proof-of-concept opens new opportunities for three-dimensional printing of hollow-core fibers incorporated with cell cultures excluding the time consuming procedure of seeding scaffolds after fabrication. The research was supported by RSF (project № 21-79-10384).

Keywords: Cell-Laden Hydrogel Fiber, Vessel, Printing.

REGULAR SESSIONS

Id-384

Nanotextured Films Encapsulating Doxorubicin Hydrochloride for Cancer Treatment

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Abstract: Creating surface textures on polymers generates novel materials with useful properties for pharmaceutical and tissue-engineering applications. Textured surfaces could mimic topographical properties of extracellular matrix, and could modulate cellular response in various ways including altering cell adhesion, proliferation, orientation, alignment, migration and morphology. Despite their advantages, these systems have not fully evaluated for pharmaceutical applications. In this study, our latest findings on textured surfaces will be presented by comparing them with our previous studies. In our previous works, we have developed a novel fabrication technique to prepare defect free nanotextured surfaces by selfassembly of the PS beads on the water-air interface and fully characterise the surfaces in terms of presence of step edges and macroscopic features on the surfaces. Afterwards nanotextured surfaces were prepared by casting hydrophobic polymers, poly(lactic-co-glycolic acid) and polycaprolactone, and the nanotextured surfaces were evaluated. In this work, we apply the method on a series of hydrophilic emulsion polycaprolactone films encapsulating an antineoplastic agent, and evaluated the films using human ovarian cancer cells. The films having 280 nm, 210 nm, and 99 nm hemispherical protrusion diameters were obtained, and then the surface topography was analysed by section and bearing analysis. Then the films are further characterized by atomic force microscopy, contact angle measurements, and fourier transform infrared spectroscopy. Cell culture studies were carried on drug loaded films including cell adhesion studies, cell viability assays (both MTT assay and trypan blue staining assay), cell apoptosis assays (both Annexin V/propidium iodide binding assay and Mitochondrial membrane potential (MMP) assay) on all drug loaded nanotextured surfaces. Cell culture the results demonstrated that nanotextured surfaces, especially surfaces with 99 nm texture diameters are better in terms of modulating cell response by cell adhesion and viability studies, and eliminating

cancer cells by the drug release. Also, all nanotextured films found to be better for eliminating cancer cells compared to 0 nm films. This work supported by The Scientific and Technological Research Council of Turkey (TÜBİTAK) [grant number 114S525]. XPS and contact angle analyses were performed at the METU Central Laboratory R&D Training and Measurement Center.

Keywords: Nanotexture, Colloidal Lithography, Ovarian Adenocarcinoma Cell, Doxorubicin Hydrochloride, Drug Delivery.

REGULAR SESSIONS

Id-387

Lateral Flow Immune Sensors for Phycotoxins: Improved Assays and New Reactants for Sensitive Detection

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Abstract: Currently, many cases of human poisoning with phycotoxins produced by phytoplankton are recorded worldwide. The extremely toxic phycotoxins microcystin-leucinearginine (MC-LR) and domoic acid (DA) secreted by cyanobacteria and microalgae, respectively, can cause serious harm to humans upon intake of contaminated food or water. MC-LR and DA induce both acute toxicity and delayed adverse effects. Poisoning is manifested by gastrointestinal and neurological symptoms and can be lethal. Therefore, rapid and sensitive techniques for monitoring phycotoxins in seafood and water are in great demand. Immunochromatographic analysis (ICA) can serve as a simple, cheap, sensitive, and specific method for rapid point-of-care control of MC-LR and DA. Despite the availability of immunoassays and some commercial kits for the determination of these phycotoxins, there is still a need to develop approaches to reduce their limit of detection (LOD). The aim of this study was the development of ICAs for the rapid determination of MC-LR and DA. For this purpose, new immunoreagents were obtained, and approaches aimed at LOD reduction, namely indirect labeling, the introduction of preincubation steps, alternative labeling, and post-analytical signal amplification were successfully applied. Thus, for MC-LR, two indirect ICAs that allowed an increase of assay sensitivity were developed. The first ICA was based on the use of gold nanoflowers instead of traditional gold nanoparticles (AuNPs) as a marker. In the second ICA, magnetic particles (MPs) were used as a label and as a carrier for horseradish peroxidase as a means of signal amplification. The developed test systems allowed a gain in assay sensitivity of up to several orders in comparison to the ICAs reported in the literature with preservation of the assay rapidity. Thus, using MPs and peroxidase signal amplification resulted in the instrumental LOD of 2 pg/mL and a cutoff of 50 pg/mL. The applicability of the ICAs to the detection of MC-

LR in fish and seafood with recoveries of 71–115% was demonstrated. For DA, nine clones of DA-specific monoclonal antibodies (MABs) were produced in Balb/c mice. The obtained MABs were characterized by the enzyme-linked immunosorbent assay, and the clone having the highest affinity to DA was selected for the development of the ICA. The ICA was performed in the direct competitive format based on the introduction of the marker to anti-DA antibodies. As a label, AuNPs were obtained and conjugated with MABs by physical absorption. The ICA was characterized by the instrumental LOD of 0.6 ng/mL and cutoff of 100 ng/mL. The assay duration was 15 min. The developed ICA was tested for the determination of DA in spiked water samples. As a result, it was shown that the ICA allowed the detection of 80–120% of DA in tap and sea water, which confirmed its effectiveness for phycotoxin determination in water samples. The solutions proposed in the study allow for the formation of promising bioanalytical platforms for the monitoring of phycotoxins, including multianalytical test systems for quantitative analysis in water and food matrices. The study was financially supported by the Russian Science Foundation (project 20-43-07001).

Keywords: Phycotoxins, Microcystin-LR, Domoic Acid, İmmunochromatographic Analysis, Food Safety.

Id-358

Hydrogen Production Using Selective Serotonin Reuptake Inhibitors in Microbial Electrolysis Cells

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Abstract: Microbial electrolysis cells (MECs) are electrochemical reactors that produce fuel hydrogen by metabolizing organic substrates available in wastewaters or biomass. Therefore, MECs were shown to be promising for small but sustainable fuel generation, biosensor development, with the added value of wastewater treatment/biological removal of environmental pollutants. Selective serotonin reuptake inhibitors (SSRIs) are a group of drugs commonly used in treatment of various disorders including depression. SSRIs drugs disposed off into public wastewaters cause biological accumulation in the long term, thus, damage to the ecosystems. In this study, hydrogen production along with methane and carbondioxide generation was analyzed using paroxetine, venlafaxine and o-desmethylvenlafaxine as substrates in single chamber MECs. MECs were inoculated with a mixed microbial culture enriched from local wastewater treatment plant using sodium acetate as the sole carbon source. Following the observation of successful hydrogen production, fate of SSRI drugs were examined in MECs. SSRIs were examined as combinations of all three drugs at 2000 ng/mL and 500 ng/mL concentrations, respectively. In the beginning of MEC operations using 2000 ng/mL of SSRI mixture, no hydrogen and methane, but carbondioxide was detected. When the concentration of the drug mixture were decreased to 500 ng/mL, hydrogen production along with methane production was observed via gas chromatography. Removal of SSRIs during MEC operation was also analyzed using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). These results showed that MECs could offer an alternative treatment method of wastewaters containing SSRI metabolites, with the added value of fuel hydrogen generation.

Keywords: Hydrogen, Microbial electrolysis cell, SSRI.

Id-369

Saccharide Interactions with Glucose-Binding Proteins

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Abstract: The aim of this study is to investigate the process of attachment of saccharide particles differing in degree of complexity to cell receptors responsible for transport of glucose across cell membrane (GLUT proteins). This phenomenon is currently taken into consideration when designing modern medicines, e.g. peptide drugs to which glucose residues are being attached thus enabling drugs to cross the barrier of cell membranes and to act in the inside of cells. The study is meant to help to understand the process of assimilation of polysaccharide nanoparticles by tumor cells. The main question about it is what is the size limitation of a particle that can be transported this way and how does the size of attached saccharide affect the efficiency of the process of binding molecules with GLUT proteins. The interactions between different analytical standard grade saccharides (oligosaccharides and polysaccharides) were measured with the use of two SPR systems using different sensing chis that were modified with several types of GLUT proteins. We obtained sensograms of different saccharides interacting with GLUT proteins which can be compared. This work has been supported by the National Science Centre, Poland, project registration number: 2017/25/N/ST8/01027.

Keywords: Surface Plasmon Resonance, Drug Delivery Systems, Drug-Cell Interactions.

Id-378

For Rapid Determination of Target Bacterium by Using Magnetic Preconcentration of Samples an Adaptable Approach for QCM System

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Abstract: Magnetic nanoparticles are of great interest to researchers due to the multitude of application areas (such as biotechnology, pharmaceutical industry, magnetic recording devices). Magnetic nanoparticles make their application areas very convenient with 10-20 nm particle distribution. In the aforementioned size-size range, each particle becomes a single domain and exhibits super-paramagnetic properties. Magnetic nanoparticles are used as adsorbents after fictionalization with various functional groups in magnetic separation processes in the field of biotechnology, for the purification of proteins and other biomolecules. The functionalized magnetic particles can be adapted to a sensor unit and the main advantage is that it is possible to separate them magnetically from the medium in a fast and simple way for subsequent detection of the target analyte. Magnetic pre-concentration of target analytes is capturing low concentrations of target analytes from real samples onto a large surface area of magnetic particles, as a result, the analyte molecules are concentrated in small geometric areas to enable sensitive detection. Pre-concentration of the target analyte is realized to minimize the effect of interference from coexisting irrelevant molecules in sample matrices, which has been explored. In addition, the use of an external magnetic field to selectively attract desired target analytes attached to magnetic conjugates onto the sensor surface is another strategy. In this study, a magnetic nanoparticle system was constructed and used for a pre-concentration system. The double-layer polymer was formed on the surface of the magnetic Fe3O4 nanoparticles, which were prepared in an easy and inexpensive way. In the dopamine solution, Fe3O4 nanoparticles is form a layer of poly(dopamine) (PDA) on the surface with a selfpolymerizing method without the need for any activation agent. Similarly, the second layer of polymeric diaminopolyethylene glycol (DAPEG) on the surface of the Fe3O4@PDA nanoparticle can automatically bind to poly(dopamine) coated surfaces without the need for any activation

agents. The prepared nanoparticle surface was activated with glutaraldehyde for covalently binding of *L. monocytogenes* specific aptamer ligand to the Fe3O4@PDA (DAPEG) nanoparticle surface. On the surface of the QCM sensor and the newly designed Fe3O4@PDA@ DAPEG nanoparticles, the DAPEG layer coated as the second layer was intended to increase the hydrophilicity of the surface of the nanoparticle and reduce non-specific relationships with the environment of the aptamer. Following pre-enrichment of target bacteria with the MP@PDA-DAPEG-Apt particles, and the obtained eluent from the magnetic system was injected flow cell of the QCM sensor for real-time determination of *L. monocytogenes* from the samples. This study was funded by the Scientific and Technological Research Council of Turkey (TUBITAK) ARDEB 1001 Grant No 119Z886.

Keywords: Rapid Determination, Magnetic Preconcentration.

Id-393

Label-free DNA Biosensor Based on Reduced Graphene Oxide Functionalized by Diazonium Chemistry

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Abstract: The development of DNA biosensors has attracted increasing attention in the biomedical field in connection with research efforts directed at gene analysis, the detection of genetic disorders, tissue matching, and potency assays. To fulfill the need of a sensitive, lowcost, and miniaturized DNA biosensor we propose an electrochemical detection platform based on reduced graphene oxide (RGO) functionalized via diazonium chemistry. In this study, commercial screen printed carbon electrodes (SPCEs) were used as the basis of our platform and were modified with graphene oxide that was electrochemically reduced (RGO/SPCE). Covalent functionalization of RGO/SPCEs was achieved by electrochemical reduction of in situ generated 4-carboxyphenil diazonium salt, which led to the electrografting of aryl-carboxyl groups on RGO surface (RGO/Ar-COOH/SPCE). Moreover, amino-modified single-stranded DNA probe was immobilized on the functionalized electrode by covalent bonding after increasing through carbodiimide chemistry the reactivity of the functional groups grafted on RGO/SPCEs. Finally, the electrodes were incubated with complementary DNA target to achieve the hybridization between the two single-stranded biomolecules. Structural characterization of SPCEs after each modification was performed by Raman spectroscopy, while the electrochemical properties of the electrodes were investigated by cyclic voltammetry and electrochemical impedance spectroscopy, carried out in 0.1 M KCl solution containing [Fe(CN)₆]³⁻/[Fe(CN)₆]⁴⁻ redox pair (1:1). The basis of hybridization detection relies on changes in the electrochemical signal produced by the binding of target DNA to the aptamer. Electrochemical measurements show a sensitive loss in electrical conductivity after immobilization of DNA probe on the graphene-based platform. Hybridization with DNA target is

detected by a decrease of Rct explained by a less negative charge density of the functionalized electrode facilitated by the diffusion of the negative $[Fe(CN)_6]^{3-/4}$ redox couple to the electrode surface. This work was supported by a grant of the Ministry of Research and Innovation, Operational Program Competitiveness Axis 1 - Section E, Program co-financed from European Regional Development Fund "Investments for your future" under the project number 154/25.11.2016, P_37_221/2015.

Keywords: DNA Hybridization, Biosensor, Electrochemistry, Reduced Graphene Oxide, Diazonium Chemistry.

Id-395

Development of a Lateral Flow Biosensor Using Gold Nanoparticle Conjugated Antibodies for Point-of-care Detection of Uropathogenic Escherichia Coli

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Abstract: Urinary tract infections (UTIs) are among the most common bacterial infections. About 60% of women at all ages and 12% of men suffer from at least one episode of UTI within their lifetime, about 25% of women exposed to recurrent UTI. Uropathogenic bacteria invading the bladder epithelial cells avoid from antibiotics and immune system. Therefore, they infect host cells repeatedly (recurrent UTI) and acquire resistant to broad-spectrum antibiotics. Among the bacteria causing UTIs, Uropathogenic Escherichia coli (UPEC) is the most prevalent bacterium (up to 90%). The detection of the UPEC usually relies on bacterial culture of urine sample. Therefore, a cost-effective and non-laborious lateral flow biosensor (LFB) will be strictly useful for the detection of UPEC more rapidly, sensitively and specifically without waiting a few days for growth of bacteria on solid media. It doesn't require complicated equipments and technical expertise that are critical parameters for point-of-care, and have long shelf life at room temperature. To the best of our knowledge, no such test is being reported or commercially available. Our objective is to develop a LFB based on antibodies conjugated on gold nanoparticles for rapid, inexpensive and easy detection of UPEC in urine samples of symptomatic subjects with UTIs. A proprietary recombinant protein was produced by cloning a gene of E. coli and then rabbit polyclonal antibody was generated against the recombinant protein. The antibody was conjugated to gold nanoparticles and placed into conjugate pad. Unconjugated antibodies and anti-rabbit IgG antibodies were immobilized on membrane as test line and control line, respectively. Analytical sensitivity of the LFB was determined by testing the recombinant protein. The addition of a sample drop onto the sample pad led to a lateral flow of the sample fluid containing antigens toward the conjugate pad where it bound to the antibody coated on gold nanoparticles. The complex then flowed to the membrane where it bound to the immobilized antibody on the test line and resulted in a red color. The flow of the sample fluid

continued toward the control line where the remaining antibody coated gold nanoparticles bound to the immobilized anti-rabbit IgG antibodies and gave a red color for each test, an indicator for a proper function of the test strip. Absorbent pad at the end of the strip sucked the fluid through the membrane to ensure a continuous flow and thus maintained a clear background. Results were observed by the naked eye within 5 min and the lower detection limit was 10 ng. In addition, lysates of *E. coli* and closely related microorganisms were evaluated with the LFB. No cross-reaction was observed. The proposed biosensor will provide a good aid in routine diagnosis of UTI caused by UPEC. Such a diagnostic approach with improved performance characteristics will allow for more accurate selection of therapy and an overall reduction in antibiotic use. Hereby, it will prevent antibiotic resistance, recurrent UTI, progression of disease and great health expenses. This study was supported by The Scientific and Technological Research Council of Turkey (TUBITAK) (Project Number: 318S049).

Keywords: Lateral Flow Biosensor, Antibody, Uropathogenic Escherichia Coli, Urinary Tract İnfections.

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Bio-Nanomaterials and Nanotechnology	378 - For Rapid Determination of Target Bacterium by Using Magnetic Preconcentration of Samples an Adaptable Approach for QCM System
Biological Response to Nanomaterials	327 - Ectopic Ostegenesis of Bioinspired Composite Scaffold with Graphene Oxide Filling and Hydroxyapatite Gradient Density
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