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**5<sup>th</sup> International Conference on  
Biomaterials and Biosensors  
(BIOMATSEN 2020)**

**October 14-20, 2020**

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**PLENARY SPEAKERS**

**Id-296**

**Are Better Medical Devices Enough to Improve Healthcare?**

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**Abstract:** Life expectancy in several highly industrialized nations (like the U.S. and Germany) has decreased over the past 2 years. This highlights a critical problem we are facing today with traditional healthcare which relies on non-responsive medical devices, extensive use of drugs, generalizations, and a reactive not proactive approach. Our current global healthcare system has reached its peak effectiveness and is clearly no longer working. This presentation will highlight the role that implantable nano sensors are making to transition from traditional medicine to a more proactive medicine that can reverse our declining life expectancies. Organ systems to be covered include bone, brain, cartilage, cardiovascular, and more. Diseases to be covered include cancer, infection, and others.

**Keywords:** Sensors, Implants.

**INVITED SPEAKERS**

**Id-315**

**Dual Stimuli-Sensitive Copolymer Hydrogels: Synthesis and Characterisation**

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**Abstract:** Dual stimuli-sensitive hydrogels, especially thermo- and pH- sensitive hydrogels have special importance for human application, because the temperature and pH value are parameters that most often change in a living organism. They may be synthesized by the polymerization of comonomers with hydrophobic and ionization functional groups, by the new monomers that respond simultaneously to both stimuli, or combining temperature sensitive polymers with polyelectrolytes. By introducing small amounts of weak acid, swelling capacity increases, and the volume phase transition temperature of copolymers is transferred to higher values. There have been numerous research with dual thermo- and pH responsive hydrogels in the controlled and self-regulated drug delivery systems, in medicine, in bioengineering, or in biotechnology fields. The aim of this work is to characterize properties of synthesized hydrogels based on thermosensitive monomer N-isopropylacrylamide and different anionic comonomers (acrylic acid and methacrylic acid). The hydrogels were synthesized by radical polymerization process with different comonomer and cross-linker content. In this work, the influence of lyophilization process on their swelling properties was examined. The synthesized poly(N-isopropylacrylamide-co-acrylic acid) p(NIPAM-AA) and poly(N-isopropylacrylamide-co-methacrylic acid) p(NIPAM-MAA) hydrogels were characterized using different instrumental techniques, as well as Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), X-ray diffraction (XRD), and differential scanning calorimetry (DSC). FTIR spectra of obtained hydrogels indicate successful polymerization process by C=C double bonds breaking. The residual reactants content during the polymerization process was determined using high pressure liquid chromatography (HPLC) method. The values of unreacted monomer and cross-linker quantities were within acceptable limits and indicate almost complete conversion of reactants during synthesis. The swelling ratio in fluids at different pH values and temperatures were analysed. Hydrogels p(NIPAM-AA) and p(NIPAM-MAA) are negatively

thermo-sensitive, because their swelling rate decreased with temperature increase. Hydrogels swollen up to the equilibrium state were lyophilized and applied in further analysis. Obtained results show that lyophilized p(NIPAM-AA) and p(NIPAM-MAA) hydrogels achieve equilibrium swelling capacity faster than non-lyophilized samples. Swelling kinetics before and after the lyophilization process, as well as swelling reversibility through 3 alternate swell-shrink cycles under the influence of temperature were investigated. A full three-level experimental design was applied in order to describe the swelling process of analysed hydrogels as a function of temperature, pH value and crosslinker content by a mathematical model. The obtained results, achievements and their importance present possibility to apply this kind of hydrogels as promising candidates for preparation of potentially suitable drug carriers, with the possibility of designing the desired properties. Republic of Serbia - Ministry of Education, Science and Technological Development, Program for Financing Scientific Research Work, ev. no. 451-03-68/2020-14/200133.

**Keywords:** Thermo and Ph-Sensitive Hydrogels, Lyophilization, N-Isopropylacrylamide, Acrylic Acid, Methacrylic Acid.

INVITED SPEAKERS

Id-320

**New Types of Amperometric Enzyme Biosensors Based on Novel Polymer Matrixes and Micro/Nanocomposites for Xenobiotics Analysis in Wastewater**

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**Abstract:** Technogenic pressure on the environment significantly affects the pollution of water resources. Especially dangerous are xenobiotics – products of the chemical and pharmaceutical industry, which negatively impact on the physiological state of living organisms and have carcinogenic properties even at very low concentrations. Conservation and restoration of water resources is a huge problem for modern society. Some xenobiotics, apart from wastewater treatment plants, are also in the surface and underground waters, because they were only partially removed in the process of the existing technological schemes for cleaning of wastewater. One of such innovations is creation of highly sensitive biosensors for analysis of the level of wastewater pollution. The studies of micro/nano-modified polymers will lead to the creation of devices with a significantly expanded range of capabilities for detecting harmful contaminants. The main aim of the research carried out was focused on the creation of new biorecognizing layers based on enzymes for analytical purposes and novel polymer matrixes and electroconductive micro/nanomaterials, which can be used for effective enzyme immobilization. Nanoparticles of noble metals and highly dispersed inorganic materials were used as nanocarriers of bioselective molecules. The synthesized novel polymer matrixes and micro/nanomaterials were used for the construction of biorecognition layers of new amperometric biosensors. Special attention was paid to study the possible advantages of nanoparticles compared with their micro-analogues, in particular, possibility of direct (mediatorless) electrochemical communication between enzymes and electrode surface in redox systems on the example of laccase. The author thanks Profs. M. Gonchar and T. Petkova and Drs. O. Smutok, O. Šauša, H. Švajdlenková, V. Boev, V. Ilcheva, and J. Ostrauskaite for fruitful collaboration in this research. This work was financially supported by the Ministry of Education and Science of Ukraine (Nos. 0118U000297 and 0119U100671). The author also

acknowledges the SAIA for scholarship in the Institute of Physics of Slovak Academy of Sciences within the National Scholarship Programme of the Slovak Republic.

**Keywords:** Amperometric Biosensor, Enzyme, Laccase, Organic-Inorganic Ureasil Polymer, Photocross-Linked Polymer.

REGULAR SESSIONS

Id-328

**Hydrogel Scaffold Formation in Turbid Medium of Biotissue via Photocrosslinking**

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**Abstract:** Formation of polymeric scaffolds in the injured area is a promising approach for augmentation of soft tissue repair. However, photocrosslinking process in the depth of scattering medium is challenging due to the small penetration depth of UV light required for exciting the photoinitiator molecules e.g., Irgacure, TPO, CQ. In order to overcome this problem we synthesized water soluble PEG-chlorin p<sub>6</sub> possessing absorption in UV and red region of spectrum within the biotissue transparency window (626-1316 nm). In situ gelation of biocompatible hyaluronic acid glycidyl methacrylate (HAGM) hydrogel is demonstrated. Water-soluble PEG-chlorin p<sub>6</sub> was prepared from chlorin p<sub>6</sub> trimethyl ester. Chlorin p<sub>6</sub> dimethyl ester was synthesized by selective hydrolysis with lithium hydroxide and then was converted to PEG derivative in 89% yield after N,N'-disuccinimidyl carbonate activation and reaction with monomethyl ether aminoPEG<sub>2000</sub>. It was purified using preparative TLC. HAGM was synthesized with 68% degree of substitution as described in. Light toxicity of PEG-chlorin p<sub>6</sub> was evaluated in vitro using human immortalized Bj-5ta fibroblasts by MTT assay. It was found, that PEG-chlorin p<sub>6</sub> irradiation (365 nm, 3 J/cm<sup>2</sup>) did not lead to the cell death up to 200 μM and with limited (more than 80% cell viability) toxicity at concentrations up to 400 μM. Therefore, photocurable composition (PCC) was produced by dissolution HAGM (16 wt%) and PEG-chlorin p<sub>6</sub> (200 μM) in normal saline with triethanolamine (0.5 wt%) as a coinitiator. Aiming to reproduce the turbid medium of living tissue, biotissue-mimicking phantoms were fabricated using agarose (Serva). Agarose (450 mg) was mixed with water (50 ml) and melted at 100 °C with vigorous

stirring until the complete dissolution of the polysaccharide. After cooling down to 65 °C, 1 ml of 1.8% low-lactose milk (Parmalat) was added for imitation of scattering properties. Then the liquid was placed in molds and cooled. Live cells (human HaCaT keratinocytes) were gently mixed with PCC immediately prior to photocrosslinking. PCC was placed in the cavities of phantoms and irradiated by 675 nm laser (250 mW/cm<sup>2</sup>, 17 min). The photocrosslinking of the entire PCC volume was demonstrated for 0.7, 1.8, 2.6, 3.6 and 4.8 mm thick phantoms. The cell-laden hydrogel samples were placed in RPMI-1640 cell culture medium and incubated at 37°C, and 5% CO<sub>2</sub> in a humidified atmosphere. The cells were stained with Calcein AM (50 μM, 10 min) and Hoechst 33342 (50 μM, 10 min) to visualize cytoplasm and cell nucleuses, respectively. Fluorescent microscopy indicated that keratinocytes were alive after 1, 3 and 5 days of incubation. In situ photocrosslinking was demonstrated in DBA/2 mice. PCC was subcutaneously injected on the dorsal surfaces of mice (3 pieces) and exposed to 675 nm light. On day 14 the scaffolds with adjacent tissue sites were taken for histological examination. The absence of an inflammatory response and crucial structural changes in the tissue confirms the biocompatibility of hydrogel. The research was supported by RSF(project№18-79-10198) in the part of scaffolds and RFBR(project№17-00-00118KOMFI) in the part of histology.

**Keywords:** Cell-Laden Hydrogels, Photocrosslinking, Turbid Medium, PEG-Chlorin P6.

REGULAR SESSIONS

Id-329

**Evaluation of Nanotextured Films Encapsulating Doxorubicin Hydrochloride for Drug Delivery Applications**

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**Abstract:** Smart systems that mimic morphological structure of extracellular matrix draw attention due to their ability to modulate cell behavior. Numerous studies demonstrated that cells respond to surface textures where changes in cellular alignment, adhesion, proliferation, and morphology are reported. Based on this background, in this work, we have fabricated nanotextured films encapsulating doxorubicin hydrochloride, and evaluated them as a new drug delivery system. By these nanotextured films, we aimed to influence the behavior of cancer cell, and at the same time manage cancer treatment. Films with hemispherical protrusion shaped surface texture were fabricated by colloidal lithography and polymer casting. Mono-layered arrays of polystyrene particles with the diameters of 99, 210, and 280 nm were used as templates, which are then transferred to polydimethylsiloxane reverse molds. Nanotextured films were obtained by casting water-in-oil stable polycaprolactone emulsions to polydimethylsiloxane molds. Non-textured films were also fabricated for control studies. After fabrication, films are characterized by atomic force microscopy, X-ray photoelectron spectroscopy, contact angle measurements, and fourier transform infrared spectroscopy. Afterwards films were further evaluated for drug delivery by encapsulation efficiency and in vitro drug release studies. Doxorubicin HCl release kinetics from nanotextured films were conducted. According to results, surface textures were successfully transferred to films. All films were found to be hydrophilic with contact angle values around 40°. Samples were found to have PDMS remaining over topography with around 3 nm thickness. Drug release studies were carried for 60 days, and within this period of time it was found that approximately 30% of the drug is released in all formulations. For the nanotextured samples, drug release profile fits to Higuchi model, whereas for non-textured films it is the first order model. Our study showed that nanotextured films could be a promising system for drug delivery. Further studies should be carried to evaluate full potential of nanotextured films for drug delivery. This work was 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. I would like to thank Professor Oya Orun and Assoc. Professor Pinar Mega Tiber for their contributions.

**Keywords:** Nanotexture, Colloidal Lithography, Doxorubicin Hydrochloride, Polycaprolactone, Drug Delivery.

POSTER SESSIONS

Id-297

**Non-linear Analysis of Deformation Behavior of HA/PLCL Porous Bio-composites**

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**Abstract:** It is well-known that the biology of bone mainly consists of the inorganic minerals, therefore bioceramics with similar constituent to that inorganic minerals of bone, e.g., hydroxyapatite  $[\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2]$  (HA) are extensively used for the scaffold fabrication. However, the major problems of HA porous material such as insufficient mechanical strength and brittleness limit its application especially in hard tissue implant. Hence, biodegradable synthetic polymers (biopolymer) are commonly used as composite materials to enhance the mechanical properties of HA porous scaffold. In this present work, HA based scaffold incorporated biopolymer namely, Poly(L-Lactide-co- $\epsilon$ -Caprolactone) (PLCL) is developed in an attempt to overcome the problem. Further, two theoretical models are developed to express the non-linearity of the load-displacement curve obtained from the three-point bending test. Finally, the surface morphology of this material is observed using the Scanning Electron Microscopy (SEM). HA/PLCL material is observed to have non-linear deformation where Model I depicts better accuracy compared to Model II with 7.76% error while 11.61% error for Model II. However, due to load limitation of Model I, Model II seems to have a better prediction on the load-displacement curve. The morphological structure of HA/PLCL shows two layers of interconnecting pores where the addition of polymer has enhanced the mechanical properties by providing ductility.

**Keywords:** Biomaterial, Non-Linear Deformation, Mechanical Behavior.

POSTER SESSIONS

Id-321

**Investigating the Network Properties of Polymer Matrixes for Controlling a Functionality of Laccase-based Amperometric Biosensors**

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**Abstract:** Application of polymer materials as holding matrixes of immobilized enzyme is an innovative approach in a construction of the non-mediated laccase-based biosensors of the third generation. The constructed biosensor based on the ureasil/As<sub>2</sub>S<sub>3</sub> composite was characterized by a very high sensitivity, but a weak point of the biosensor was very strong unexpected electrochemical noise at chronoamperometric measurement. At the same time, new perspectives of the ureasil-based polymers for construction of amperometric enzyme biosensors were further found. In particular, a correlation between the network properties of the biosensor sensing layers (e.g., free-volume  $V_h$  at glass transition temperature  $T_g$  and coefficients for the thermal expansion of free-volume voids  $\alpha_{F1}$ ,  $\alpha_{F2}$  as well as their difference ( $\alpha_{F2} - \alpha_{F1}$ ), and swellability or crosslinked density) based on the pure ureasil and ureasil/As<sub>2</sub>S<sub>3</sub> composites of different history (fresh and aged samples) and biosensor characteristics (e.g., a maximal current at substrate saturation  $I_{max}$ , apparent Michaelis-Menten constant  $K_M^{app}$  to ABTS chosen as a substrate, the slope of the calibration curve  $B$ , and the sensitivity of bioelectrodes obtained by means of cycle voltammetry and chronoamperometric analysis) was established. On the other hand, vegetable oil-based photopolymers could be also used as a holding matrix in biosensors. Recently, laccase-based amperometric enzyme biosensors of the third generation for analysis of phenol derivatives was constructed using graphite rods (type RW001) as working electrodes and the photocross-linked polymers as a matrix. Such matrix consisted of epoxidized

linseed oil (ELO), bisphenol A diglycidyl ether (RD) as reactive diluent and 50% mixture of triarylsulfonium hexafluorophosphate in propylene carbonate (PI) as photoinitiator. The synthesis was made by the reaction of ELO and 10 mol.% or 30 mol.% of RD, using 3 mol.% of PI (ELO/10RD and ELO/30RD, respectively). The holding matrixes were used for an immobilization of commercial laccase from the fungus *Trametes versicolor*. The network properties of the polymer matrixes, holding biosensing element, were studied by means of positron annihilation lifetime spectroscopy (PALS) and swelling measurements. The amperometric enzyme biosensor parameters were evaluated using cyclic voltamperometry and chronoamperometric analysis. A correlation between the constructed biosensor parameters and microscopical free volume of the biosensor holding matrixes was also established. Thus, as a result of the research carried out, one may suggest about finding a possible master parameter for controlling a functionality of amperometric enzyme biosensors. This work was supported in part by the Ministry of Education and Science of Ukraine (Nos. 0117U007142, 0118U000297, and 0119U100671), Slovak Grant Agency VEGA (Nos. 2/0127/17 and 2/0157/17), Slovak Research and Development Agency (No. APVV-16-0369), and NSF of the Bulgarian Ministry of Education (No. FNI-DN09/12-2016.). T.K. and Y.K. also acknowledge the SAIA for scholarship in the IPSAS within the National Scholarship Programme of the Slovak Republic.

**Keywords:** Organic-inorganic Ureasil Polymer, Photocross-linked Polymer, Positron Annihilation, Free Volume, Swelling.

## POSTER SESSIONS

**Id-345**

### **Laccases in Biocatalysis and Modification of Biomaterials**

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**Abstract:** Laccase (benzenediol: oxygen oxidoreductase, EC 1.10.3.2) is a multi-copper-containing enzyme which performs one-electron oxidation of various substrates such as diphenols, methoxy-substituted monophenols, as well as aromatic and aliphatic amines to form radicals and at the same time reduces molecular oxygen to water. Laccase-generated radicals undergo a number of non-enzymatic reactions, which have many potential applications. In the present contribution, applications of laccases in biocatalysis and modification of biomaterials are presented. In biocatalysis, laccase-catalysed the synthesis of potent bioactive compounds (mainly antioxidants) through homomolecular coupling and the efficacy of the compounds was assessed in-vitro and ex-vivo on human skin cells (keratinocytes and fibroblasts). In the modification of biomaterials, the enzyme was used 1. to develop green processes for the surface functionalization of lignocellulose materials mainly for improving hygienic properties (hydrophobicity and antimicrobial properties), and 2. to modify food biopolymers for the production of hydrogels and functional ingredients for gluten-free products. Laccase-catalysed dimerization of phenolic compounds such as ferulic acid, 2,6-dimethoxyphenol, 3-hydroxytyrosol, caffeic acid and silybin led to the production of dimers with high antioxidant capacity in comparison to the corresponding monomeric substrates. The monomers were linked mainly through C-C linkages with the  $\beta$ -5 and  $\beta$ - $\beta$  linkages predominant in phenolic acids. In the modification of lignocellulose materials, fluorophenols and alkylamines were grafted to improve hydrophobicity while tannins and antifungal agents were grafted to improve antimicrobial properties. Modelling of the grafting reactions showed that functional molecules were coupled to guaiacylglycerol  $\beta$ -guaiacyl ether and dibenzodioxocin mainly through 5-5 linkages while the molecules were coupled to syringylglycerol  $\beta$ -guaiacyl ether through 4-O-5 linkages. Modification of food biopolymers improved properties of gluten-free bread and hydrogels produced from underutilised food ingredients. The processes developed have implications in various industries mainly the health and nutraceutical industries and the wood and paper industries.

**Keywords:** Laccase, Lignocellulose Materials, Surface Functionalisation, Biocatalysis.

**ALL SUBMISSIONS & TOPICS**

<b>Topic</b>	<b>Submission</b>
<b>Biomaterials</b>	Id 328 - Hydrogel Scaffold Formation in Turbid Medium of Biotissue via Photocrosslinking
	Id 345 - Laccases in Biocatalysis and Modification of Biomaterials
<b>Advanced Functional Materials</b>	Id 315 - Dual Stimuli-sensitive Copolymer Hydrogels: Synthesis and Characterisation
	Id 321 - Investigating the Network Properties of Polymer Matrixes for Controlling a Functionality of Laccase-based Amperometric Biosensors
<b>Modeling of Behavior Biomaterials</b>	Id 297 - Non-linear Analysis of Deformation Behavior of HA/PLCL Porous Biocomposites
<b>Nanobiomaterials</b>	Id 329 - Evaluation of Nanotextured Films Encapsulating Doxorubicin Hydrochloride for Drug Delivery Applications
<b>Electrochemical</b>	Id 320 - New Types of Amperometric Enzyme Biosensors Based on Novel Polymer Matrixes and Micro/nanocomposites for Xenobiotics Analysis in Wastewater
<b>Nanobiosensors and Nanotechnology</b>	Id 296 - Are Better Medical Devices Enough to Improve Healthcare ?