1ST ON-LINE WORKSHOP

SURFACE ENGINEERING FOR BIOMEDICAL APPLICATIONS

ENGINEERING WITH A MEDICAL PERSPECTIVE: FUTURE BEGINS TODAY

11 NOVEMBER 2020

BULGARIA

PROGRAM

ABSTRACTS

Editors: Albena Daskalova, Teodora Valova

BULGARIAN ACADEMY OF SCIENCES

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The Institute of Electronics carries out research and educational activities in the fields of Physical Electronics, Quantum Electronics and Radiophysics. The research is focused on: studying the processes of generation and control of beams of electrons, ions and photons, and especially on their interaction with matter; development of experimental and industrial equipment for surface modification, thin-film deposition and analysis; welding and melting of metals; low-temperature plasma, and plasma-chemical processes; plasma-assisted formation of thin films and coatings; interaction of laser radiation with matter and thin-film formation; development of laser sources and systems for spectroscopy, metrology, modification and analysis of materials; non-linear optical phenomena in fibers and semiconductors; generation and propagation of electromagnetic waves; non-linear phenomena in a wide MW-frequency range; scattering of electromagnetic waves from homogeneous and inhomogeneous media, signal detection, data acquisition and processing; development of electronic systems for the MW and optical ranges; systems for remote sounding of the atmosphere and sending of the sea and Earth surface.

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Medical University - Pleven, established in 1974, is one of the most dynamic and advanced higher medical educational institutions in Bulgaria. The University consists of 4 faculties - Faculty of Medicine, Faculty of Pharmacy, Faculty of Public health, Faculty of Health care. Medical College and Department of Language and Specialized Training are administrative units of MU-Pleven.

Today, combining traditions of the past with the present possibilities, the University incorporates educational and therapeutic facilities, contemporary pre-clinical base, University Hospital and clinics in all major medical fields, as well as a large number of specialized clinics and research units with modern diagnostic and therapeutic equipment. These are efficiently used to treat patients, train students, trainee doctors, post-graduates as well as for research work.

Medical University – Pleven is growing and developing very fast, with an emphasis on implementing advanced technologies in education and clinical practice. The medical school has two robotic systems for performing robot-assisted surgery with simulators for training and research. This ranks MU-Pleven among the leading European educational centers in robotics surgery. The medical school is advancing the practice of robotic surgery, performing Live Surgery in 3D format at modern Telecommunication Endoscopic Center. This facility, which houses its own dedicated experimental operating room, is an unique emblem of the MU-Pleven within the country and abroad.

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1ST ON-LINE WORKSHOP
SURFACE **E**NGINEERING FOR **B**IOMEDICAL **A**PPLICATIONS
11 November 2020, Bulgaria

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INVITED SPEAKERS

Prof. Amelia Almeida, Technical University of Lisbon, Lisbon, Portugal
Prof. Vera Marinova, Institute of Optical Materials and Technologies – Bulgarian Academy of Sciences
Prof. Miran Mozetič, Jozef Stefan Institute, Ljubljana, Slovenia
Prof. Endzhe Matykina, Universidad Complutense de Madrid, Madrid, Spain
Prof. Dr. Slavcho Tomov, MD, PhD, DSc, Medical University of Pleven
Prof. Grigor Gorchev, MD, PhD, DSc, Honorary Rector of the Medical University, Pleven

MAIN SCIENTIFIC TOPICS:

- Bioengineering and fundamental applications
- New materials and techniques for biomedical applications
- Application of modern engineering technologies in the medicine

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PROGRAM

BG TIME	LECTURER	PASSWORD
08:30 – 9:15	Establishing of Connection Administration & Technical Remarks Opening Remarks:	
09:15 – 09:30	***Academician Prof. Bogdan Petrunov, MD, PhD, DSc,*** ***Chairman of the Board of Trustees of MU-Pleven,*** ***Prof. Slavcho Tomov, MD, PhD, DSc, MU-Pleven and*** ***Prof. Petar Petrov, DSc, IE, BAS***	
I panel: BIOENGINEERING AND FUNDAMENTAL APPLICATIONS **Chaired by: *Chief Assist. Prof.* Stefan Valkov, PhD** ***Institute of Electronics, BAS***		
	Oral presentations	
09:30 – 09:40	***1. Influence of femtosecond laser processing parameters on surface morphology and wettability properties of polylactic acid (PLA)*** ***Assoc. Prof. Albena Daskalova, PhD*** ***Institute of Electronics, BAS***	
09:40 – 09:50	***2. Ultra-short laser modification of chitosan/ silver nanoparticles (AgNPs) thin films for potential antimicrobial applications*** ***Assoc. Prof. Ekaterina Iordanova, PhD*** ***Institute of Solid State Physics, BAS***	
09:50 – 10:00	***3. Impact of base pressure, post annealing and ageing on electrical properties of silver nanofilms*** ***Shiva Udachan*** ***Rani Channamma University, Karnataka, India***	
10:00 – 10:10	***4. Enhanced acetone-sensing properties of ZnO–noble metals composite nanostructures*** ***Assoc. Prof. Anna Dikovska, PhD*** ***Institute of Electronics, BAS***	
10:10 – 10:15	***5‘ Technical Brake***	
10:15 – 10:25	***5. Effect of interfacial compatibilization on PLA/Mg biocomposites for bioresorbable implants*** ***Meriam Ben Abdeljawad*** ***Laboratory of Polymeric and Composite Materials (SMPC), Center of Innovation and Research in Materials and Polymers (CIRMAP), University of Mons, Belgium***	

10:25 – 10:35	**6.** ***Atmospheric plasma: a simple way to improve the interface between polysaccharides and polyesters*** ***Xavier Carette*** ***Laboratory of Polymeric and Composite Materials (LPCM), University of Mons, Belgium***
10:35 – 10:45	**7.** ***A femtosecond laser-based strategy to modulate the race for the surface: micro/nanostructured ceramics surfaces to improve osteogenic differentiation and diminish bacterial adhesion*** ***Angela Carvalho[1,2]*** ***[1]i3S - Instituto de Investigação e Inovação em Saúde, U. Porto, Portugal; [2]INEB - Instituto de Engenharia Biomédica, U. Porto, Portugal***
10:45 – 10:50	***5' Technical Brake***
	Plenary lectures
10:50 – 11:20	**8.** ***Femtosecond laser surface treatment for application in biomedicine*** ***Prof. Rui Vilar*** ***Technical University of Lisbon, Lisbon, Portugal***
11:20 – 11:50	**9.** ***Laser synthesis of the new Ti-based alloys for prosthetic application*** ***Prof. Amelia Almeida*** ***Technical University of Lisbon, Lisbon, Portugal***
11:50 – 13:00	***Lunch Break***
II panel: NEW MATERIALS AND TECHNIQUES FOR BIOMEDICAL APPLICATIONS **Chaired by: *Assoc. Prof. Albena Daskalova, PhD*** ***Institute of Electronics, BAS***	
	Plenary lectures
13:00 – 13:30	**10.** ***Hybrid functionalized biomaterials for tissue engineering*** ***Prof. Endzhe Matykina*** ***Departamento de Ingenieria Química y de Materiales, Facultad de Ciencias Químicas, Universidad Complutense, 28040 Madrid, Spain***
13:30-14:00	**11.** ***Graphene potential for optoelectronic and biomedical application*** ***Prof. Vera Marinova*** ***Institute of Optical Materials and Technologies (IOMT-BAS), Sofia 1113, Bulgaria***
14:00 – 14:05	***5' Technical Brake***
	Oral presentations

14:05 – 14:15	**12.** ***Structure and biocompatibility of PVD deposited TiN/TiO_2 coatings on electron beam treated Ti6Al4V alloy*** ***Assoc. Prof. Maria Nikolova, PhD*** ***Dept. of Material Science and Technology, University of Ruse "A. Kanchev", Ruse, Bulgaria***
14:15 – 14:25	**13.** ***The altered behavior of mesenchymal stem cells on 2D collagen matrices in oxidative environment*** ***Svetoslava Stoycheva*** ***Department of Biochemistry, Medical University-Pleven, Bulgaria***
14:25 – 14:35	**14.** ***Electron-beam surface alloying of Ti substrate with Ta films*** ***Chief Assist. Prof. Stefan Valkov, PhD*** ***Institute of Electronics, BAS***
14:35 – 14:45	**15.** ***Roughness and mechanical properties of electron beam surface modified and TiN/TiO_2 coated Ti6Al4V alloy for biomedical application*** ***Assoc. Prof. Maria Nikolova, PhD*** ***Dept. of Material Science and Technology, University of Ruse "A. Kanchev", Ruse, Bulgaria***
14:45 – 14:55	**16.** ***Surface modification of Co-Cr-Mo alloys by electron beam treatment*** ***Chief Assist. Prof. Stefan Valkov, PhD*** ***Institute of Electronics, BAS***
14:55-15:05	**17.** ***Biogenic ferroxides for application in electronics, biomedicine and biotechnology derived from leptothrix bacteria*** ***Ralitsa Angelova, PhD*** ***Institute of Electronics, BAS***
15:05 – 15:10	***5 ' Technical Brake***

III panel: APPLICATION OF MODERN ENGINEERING TECHNOLOGIES IN THE MEDICINE

Chaired by: Assoc. Prof. Nadia Veleva, PhD

Medical University - Pleven

Plenary lectures

15:10 – 15:40	**18.** ***Haemo-compatibility of plasma-treated vascular implants*** ***Prof. Miran Mozetič*** ***Department of Surface Engineering, Jozef Stefan Institute, Ljubljana, Slovenia***
15:40 – 16:10	**19.** ***How robotics has changed modern surgery*** ***Prof. Slavcho Tomov, MD, PhD, DSc*** ***Medical University – Pleven, Bulgaria***
16:10 – 16:40	**20.** ***Comparative analysis of robot-assisted and abdominal radical hysterectomy for patients with cervical cancer***

Prof. Grigor Gorchev, MD, PhD, DSc, Corresponding member of Bulgarian AS

16:40 – 16:45 ***5 ' Technical Brake***

Oral presentations

16:45 – 16:55
21. Investigation of the deviation during the information transfer from the prosthetic field to the laboratory scanners
Hristina Galeva
Faculty of Dental medicine, Medical University, Sofia, Bulgaria

16:55 – 17:05
22. Effect of deposition parameters on the structural and mechanical stability of Ta-based coatings deposited on polymers for biomedical applications
Assoc. Prof. Nikolai Donkov, PhD
Institute of Electronics, BAS

17:05 – 17:15
23. The digital axiograph – a novel tool in bruxism prevention
Iva Taneva
Faculty of Dental medicine, Medical University, Sofia, Bulgaria

17:15 – 17:25
24. All-optically controlled density of alkali atom vapors for biomagnetic sensing applications
Chief Assist. Prof. Stoyan Tsvetkov, PhD
Institute of Electronics, BAS

17:25 – 17:35
25. Genetic landscape of primary lung adenocarcinoma - single institution experience
Assoc. Prof. Nataliya Chilingirova, MD, PhD
Science and Research Institute, Medical University – Pleven, Bulgaria

17:35 – 17:40 ***5 ' Technical Brake***

17:40 – 17:50
26. Atmospheric particulate matter pollution over residential urban areas during COVID-19 Quarantine
Prof. Ivan Nedkov, DSc
Institute of Electronics, BAS

17:50 – 18:00
27. 3D bioprintinng of vascularised bone tissue: current advances and challenges
Vladislav Nankov, University Scientific Research Laboratory, Medical University – Pleven, Bulgaria

18:00 – 18:10
28. Stereotactic vacuum aspiration biopsy of the breast – current indications and technical challenges
Prof. Dobromir Dimitrov, MD, PhD

	Medical University – Pleven, Bulgaria
18:10 – 18:20	***29. Clinical application of Next generation sequencing technology*** ***Prof. Katia Kovacheva, MD, PhD*** ***Medical University – Pleven, Bulgaria***
18:20	Closing Remarks

Plenary Lectures

HYBRID FUNCTIONALIZED BIOMATERIALS FOR TISSUE ENGINEERING

E. Matykina[1], *A. Santos-Coquillat*[1,2], *E. Martinez-Campos*[3], *L. Moreno*[1], *R. Arrabal*[1], *M. Mohedano*[1], *A. Gallardo*[3], *J. Rodríguez-Hernández*[3]

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State-of-the-art strategies for design and fabrication of smart biomaterials for tissue engineering address metallic, ceramic, polymeric and cell components individually and in various combinations, with the aim to tailor the therapeutic strategy to the needs of patients of specific age groups and medical conditions. In this regard, a concept of a hybrid biomaterial combining a metallic core with bioceramic and polymeric surface layers with hierarchical organization and drug-eluting capacity has emerged in order to achieve a synergy of biological activity, controlled degradation rate and mechanical reinforcement.

The lecture outlines key technologies and steps to design hybrid smart and multifunctional biomaterials. Those include *additive manufacturing* of metallic cores, such as titanium and magnesium alloys for permanent and temporary, i.e. biodegradable, implant applications. The three levels of hierarchical surface functionalization concern with i) *in situ* modification of the core material, incorporating bioactive inorganic species and phases by means of electrochemical treatments based on *anodizing*; ii) post-treatment application of polymer layers with specific topography by means of *breath figure* approach and iii) application of a cellular component, previously differentiated or co-cultured, by means of cell sheet engineering using hydrogels.

The state of the art of loading of growth factors and/or active pharmaceutical agents onto the porous ceramic and/or polymeric component of a hybrid biomaterial, such as direct incorporation and mesoporous nanocontainer-assisted incorporation and the relevant smart unloading mechanisms and release rates are also highlighted.

GRAPHENE POTENTIAL FOR OPTOELECTRONIC AND BIOMEDICAL APPLICATIONS

Vera Marinova

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Graphene, due to its extraordinary properties as high transparency, very high electrical conductivity and excellent flexibility attract an enormous interest in many areas of research and application, including optoelectronics, energy, photonics as well as in biomedical field [1]. Graphene possesses high transparency in the visible and near infrared range- the spectral interval, important for biomedical monitoring and diagnostic. In addition, graphene offers simultaneously mechanical compatibility (its Young's modulus ranges between 20–40 GPa, while the tensile strength lies between 15–520 MPa, which places graphene amongst the strongest materials), cell adhesion and low toxicity properties that opens opportunity in sensing and drug delivery. Biocompatibility is another important benefit, since graphene sp2 carbon surface allows strong, non-destructive, interfacial interactions at a cellular level. Moreover, graphene surface cam be optimized to be superhydrophobic holding self-cleaning features that can significantly benefit antibacterial activity for virus prevention and filtration [2]. All above properties, accompanied by high flexibility, makes graphene the ideal component for flexible biomedical devices and implants, since it is able to accommodate on the surrounding biological tissue without experiencing stress or fatigue.

In this talk the latest developments of the graphene and graphene derivatives into optoelectronic and biomedical fields will be presented and analyzed. Starting from graphene synthesis strategies, functionalization, characterizations, examples of graphene-based devices as Graphene Field Effect Transistor-based Biosensors and Graphene-based spatial light modulators operating at near infrared spectral range will be presented and demonstrated [3]. Finally, new trends and guidelines for future developments will be discussed.

References:

[1] Parveen Kumar, Peipei Huo, Rongzhao Zhang, and Bo Liu "Antibacterial Properties of Graphene-Based Nanomaterials" Nanomaterials 9, 737 (2019)

[2] Hong Zhong, Zhaoran Zhu, Jing Lin, Chi Fai Cheung, Vivien L. Lu, Feng Yan, Ching-Yuen Chan and Guijun Li "Reusable and Recyclable Graphene Masks with Outstanding Superhydrophobic and Photothermal Performances" ACS Nano (2020) https://dx.doi.org/10.1021/acsnano.0c02250

[3] Marinova, V., Lin, S. H., Petrov, S., Chen, M. S., Lin, Y. H., and Hsu, K.Y.: Graphene-based spatial light modulator operating at near infrared spectral range. Applied Surface Science, 472, 2-9 (2019)

HAEMO-COMPATIBILITY OF PLASMA-TREATED VASCULAR IMPLANTS

Miran Mozetič,
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Jamova cesta 39, 1000 Ljubljana, Slovenia

Cardio-vascular diseases represent the major cause of death in developed countries. Vascular stents are a common treatment for advanced peripheral and cerebrovascular disease. Badly damaged blood vessels, however, are replaced with synthetic ones, often made from polymers such as polyethylene terephthalate (PET). The polymeric vascular grafts are often tubes of woven fabrics. Such grafts remain stable over decades and provide optimal permeability as well as mechanical properties. The surface, properties, however, are far from being adequate. The blood constituents interact with the surface of polymer causing numerous complications which are often reflected in thrombosis or restenosis. The complications are linked to irreversible adsorption of constituents including but not limited to blood proteins, vesicles and exosomes. Several coatings have been proposed to suppress such effects but none provides optimal solution. An alternative to deposition of anti-thrombogenic coatings is treatment of bare polymeric vascular grafts with non-equilibrium gaseous plasma. The plasma treatment causes transformation of the surface wettability from moderately hydrophobic to super-hydrophilic surface finish. Such a surface finish is a result of mild chemical etching and functionalization. The etching causes rich morphology on the sub-micrometer scale, and functionalization the formation of highly polar surface groups. Both effects cause the capillary effect so that the gaps between the nanofeatures fill with most polar constituent of human blood – water. The interaction between the blood platelets is thus suppressed so the platelets do not activate on the surface, release factors etc what otherwise triggers transformation of fibrinogen to fibrin fibers and initiate thrombus formation. The technique will be presented and explained to some details.

HOW ROBOTICS HAS CHANGED MODERN SURGERY

Slavcho Tomov, MD, PhD, DSc

Professor at Gynecology Oncology Department
Saint Marina University Hospital Pleven, Bulgaria
Rector of Medical University Pleven, Bulgaria

In 1985, the first surgical application of robotic technology was described when an industrial robotic arm was modified to perform a brain biopsy. In January 2008 the first robotic surgical system arrived in Bulgaria. At present, three robotic systems are available at Medical University Pleven, Bulgaria. For 11 years, more than 1,100 gynaecologic robotic operations have been performed. After the first robot-assisted anterior rectal resection in 2014 was made, more than 50 robotic operations in general surgery and 150 robot-assisted urologic procedures were performed.

In the beginning there was open surgery, followed by laparoscopic and robotic surgery. The next era in surgery will belong to digital surgery. It is a kind of symbiosis between natural and artificial intelligence. The most important question is: Are we prepared for this challenge?

ANALYSIS OF ABDOMINAL VERSUS ROBOTIC RADICAL HYSTERECTOMIES FOR PATIENTS WITH CERVICAL CANCER

Grigor Gorchev, MD, PhD, DSc,

Corresponding member of Bulgarian AS
Professor at Gynecology Oncology Department
Saint Marina University Hospital Pleven, Bulgaria

OBJECTIVE: To assess and compare overall survival (OS) and disease-free survival (DFS) as well as perioperative outcomes for women with cervical cancer, for whom robotic or abdominal radical hysterectomy was performed.

METHODS: We analyzed retrospectively women with histologically diagnosed cervical cancer operated by the two surgical methods for the period 2008 - 2018. The data analyzed includes patient and tumor characteristics, perioperative outcomes and disease status. The Kaplan-Meier method and Cox regression analysis were performed in regard to OS and DFS.

RESULTS: There were 1347 patients (341 robotic and 1006 abdominal) included in the study. The median follow-up time for the robotic group was 5.24 years and for the laparotomy group - 4.32 ($p<0.001$). The Kaplan-Meier analysis demonstrated that robotic cohort had significantly higher survival rate compared to the abdominal group (Cancer specific death 8.5% vs. 16.5% respectively). The mean time to recurrence did not differ significantly by the two types of surgery ($p=0.495$). Cox multivariate regression showed no statistically significant impact of surgical approach on OS or DFS. Women in the robotic group had significantly shorter median hospital stay (7 vs. 11 days, $p<0.001$), higher postoperative hemoglobin (116 vs. 108 g/l, $p<0.001$) and less blood transfusions (7.3% vs. 21.5%, $p<0.001$). Their overall incidence of postoperative complications was also lower (2.1% vs. 9.4%, $p<0.001$).

CONCLUSION: The results from this retrospective study demonstrate no significant difference in OS and DFS between RRH and ARH for women with cervical cancer. According to our data, RRH does not lead to worse oncologic outcomes and is associated with better perioperative outcomes. Based on the obtained results and in regard to all stages of CC, we find robotic surgery a safe and sustainable superior technique compared to laparotomy. We consider as a best treatment option the more personalized surgery, relied on individual approach to the patient.

Oral Presentations

INFLUENCE OF FEMTOSECOND LASER PROCESSING PARAMETERS ON SURFACE MORPHOLOGY AND WETTABILITY PROPERTIES OF POLYLACTIC ACID (PLA)

Albena Daskalova[1], *Liliya Angelova*[1], *Rosica Mincheva*[2], *Xavier Carrete*[2], *Anton Trifonov*[3], *Ivan Buchvarov*[3]

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Regenerative medicine and tissue engineering in particular, represent the basis of today's and future medicine. Poly-lactic acid (PLA) is a very attractive newly emerging material with the potential to become a basic one, as it is a biocompatible and biodegradable synthetic polymer, whose morphological qualities can be further improved. Ultra-short pulse laser treatment is a non-invasive method that perfectly fits into the idea of optimizing the surface properties of cell-engineered matrices, as it can offer strictly controlled porosity of the biomaterial treated, tailored specifically for implantation. This topography-reforming technique successfully overcomes the limitations associated with the application of other physical and chemical methods as the side effects caused by the interaction of fs laser pulses with biological tissues are minimized.

The main goal of this study is to acquire initial knowledge of the porosity and morphology of PLA samples, after ultra-fast laser modification for future preparation and design of porous PLA-based cell matrices, which is the basis for creating effective bio-interfaces between the tissues of the recipient and the foreign implant. For this purpose, fs modified microstructured polymer scaffolds were investigated by SEM, EDX and FTIR. In order to elucidate the hydrophilicity of the treated surface compared to non-treated one, wettability measurements were also performed. Topography modification of the PLA substrates could essentially improve bioactivity properties of this material, which, after proper optimization of laser parameters, could make its biomedical applications even more successful.

Keywords: PLA, Femtosecond Laser Processing, Bio-interfaces, Tissue engineering, Cell scaffolds

Acknowledgements: Authors acknowledge project KP-06-H 38/5 (2019-2022), “Functionalization of 3D printed fibrous scaffolds via femtosecond laser patterning” and National Scientific Programe “Young scientists and postdoctorants” PMC №577/17.08.2018 (2020), “Preparation and design of porous polymer-based bio-interfaces and cell matrices by laser - induced microstructuring” for the support of this study.

ULTRA-SHORT LASER MODIFICATION OF CHITOSAN/ SILVER NANOPARTICLES (AgNPs) THIN FILMS FOR POTENTIAL ANTIMICROBIAL APPLICATIONS

G. Yankov[1], E. Iordanova[1], A. Daskalova[2], A. Dikovska[2], L. Angelova[2], D. Aceti[2], E. Filipov[2], G. Stanev[2], B. Calin[3], M. Zamfirescu[3]

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In the last years, there have been increasingly rapid advances of using biopolymers in tissue engineering for regenerative medicine purposes. The growing demand for preparing materials with desired physical, biological and mechanical properties requires active investigations in the field of tissue engineering [1,2]. Among the variety of biopolymers chitosan has been shown to be transcendent material due to its distinguished properties such as biocompatibility, biodegradability and wide range of fabrication technologies [3].

The present work is a case study of an extensive research on functionalization of 3D printed fibrous scaffolds and thin biopolymer films via laser patterning. The aim of the current study is to investigate the optical properties of biopolymer films in specimen of chitosan and chitosan with additives of silver nanoparticles.

The samples are divided into two groups -treated and non-treated by laser irradiation. Each group has samples with chitosan and composite of chitosan and 10% of silver nanoparticles (AgNPs). As laser sources are used nano- and femto- second laser systems. The applied wavelengths are 266, 355, 532 and 800 nm. Deviation of contact angle values is observed on treated and untreated areas. The obtained compositions are investigated by spectral analyses via spectrometer and optical microscope. Furthermore, the morphology of the samples is monitored by SEM analyses. The obtained results demonstrate the potential of the applied method to obtain diverse porous modifications in respect to applied laser parameters. The addition of AgNPs will drastically increase the antimicrobial properties of thin chitosan films and thus enhance the biocompatibility properties of the created 2D matrix.

References:
[1] M.M.Rahman, et al. Preparation and properties of biodegradable polymer/nano-hydroxyapatite bioceramic scaffold for spongy bone regeneration, J.Polym.Eng.39(2), 134–142, 2019
[2] S.H.K. SafiyyaYousaf, et al. Handbook of Tissue Engineering Scaffolds, Woodhead Publishing, United Kingdom, pp.649–672, 2019
[3] Md. M. Islam, et al. Chitosan based bioactive materials in tissue engineering applications-a review, Bioactive Materials 5, 164–183165, 2020

IMPACT OF BASE PRESSURE, POST ANNEALING AND AGEING ON ELECTRICAL PROPERTIES OF SILVER NANOFILMS

Shiva L. Udachan[1], N. H. Ayachit[2], Udachan. L. A[3], Shivakumar Siddanna[4] , Shrishail. S. Kolkundi[5], Ramya S.[6]*

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Silver Nanoparticles (AgNPs) have excellent potential applications in medical, environment, electronics, dielectrics, and optical solar cell application. It is urgently required to perform an extensive research of various AgNPs and its derivatives for multiple applications. Silver and silver oxide nanoparticles have potential applications in fields of nanosicence and technology. Silver thin films can achieve unique optical and electrical properties and can have better performance relative to other metal films in optical applications. Silver layers are ideal reflecting and conducting electrodes for thin film solar cells. This paper reports the growth of nanostructure of silver nanofilms prepared by thermal evaporation in vacuum. In this work, we focused on the effect of deposition pressure, annealing and ageing on electrical properties of silver films. The resistance of the sample was measured by the standard Four Probe Technique. The electrical data pertaining to the impact of pressure and annealing was analysed with the help of Mayadas-Shatzkes theory.

Keywords: Electrical resistivity, Annealing, Ageing, Pressure and Mayadas-Shatzkes theory.

ENHANCED ACETONE-SENSING PROPERTIES OF ZnO–NOBLE METALS COMPOSITE NANOSTRUCTURES

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It is well known that the human body exhales highly humidified gases consisting of over 870 compounds occurring at concentrations from ppt to ppm [1]. In this light, analyzing the human breath is considered as a new strategy of evaluating human body's physiological state. Various exhaled gases, such as ammonia, hydrogen sulfide, nitric oxide, volatile organic compounds etc. have been recognized as biomarkers for diseases and metabolic processes [2]. Acetone is identified as a specific biomarker diagnosing human type-I diabetes, since its concentration in the exhaled breath of a diabetic patient is higher than that in healthy people. It was reported that the acetone concentration in the breath of healthy people should be below 1 ppm, while it was found to exceed 1.8 ppm in diabetic patients [1,2]. Therefore, many attempts have been made to develop a highly sensitive acetone gas sensor. Metal oxide semiconductors are the most popular sensing materials for acetone detection due to their higher acetone-sensing response compared to other materials.
In this work, highly porous composite nanostructures consisting of ZnO and noble metals were fabricated by pulsed laser deposition in air at atmospheric pressure in view of gas sensor application. Under irradiation with UV light, the response to acetone was studied of pure and composite sensor elements (Pd-, PdAg- and Ag-ZnO). Also, the effect was investigated of simultaneous UV and red light irradiation on the sensor elements' response and response and recovery times. The results indicate that the PdAg-doped ZnO sample exhibits a significantly enhanced gas-sensing performance to acetone under simultaneous UV and red light irradiation. Detection of a concentration below 1 ppm of acetone under UV and red light irradiation was demonstrated.

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EFFECT OF INTERFACIAL COMPATIBILIZATION ON PLA/Mg BIOCOMPOSITES FOR BIORESORBABLE IMPLANTS

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In the field of osteosynthesis implants, biodegradable composites have shown to be promising candidates by combining both good biocompatibility and low degradation rate with suitable mechanical properties [1]. In this regard, due to its biodegradability, biocompatibility and thermoplastic processability that can offer potential to biomedical applications, polylactide (PLA) is deeply investigated [2,3]. However, PLA devices suffer from inactive interaction with bone tissue because of inflammatory response to acid products during the latest stage of implantation [4] and poor osteoconductivity with insufficient mechanical properties which limit its further application in bone healing [5]. To counterbalance the aforementioned issues, Mg was incorporated. Indeed, it has more similar mechanical performances to those of cortical bone compared to any other metallic or polymer. Moreover, Mg orthopaedic implants have presented attractive abilities of osteoconductivity and osseointegration [5]. The interfacial interaction between the hydrophobic PLA matrix and the hydrophilic Mg particles compromise the final properties of the implant. For this purpose, an amphiphilic poly (ethylene oxide-*b*-L, L-lactide) block copolymer was used to improve the PLA/Mg interface issue.

The biodegradability behaviour, bioactivity and corrosion aspect, mechanical performances during the degradation process in Simulated Body Fluid (SBF) and cell culture behaviour were studied. Initial SEM images of the composites revealed that the addition of the copolymer led to a smoother and homogeneous surface with interesting PLA/Mg adhesion. The presence of copolymer has also shown a positive effect on the composite wettability. Concerning the mechanical properties, the DMA results showed an improvement of the composites stiffness in function of the Mg incorporation. The best result was reached at 10 wt.% of filler. However, a decrease in the storage modulus was observed by the copolymer addition because of the plasticizing PEO effect. Interestingly, the fact that the tan δ peak width at mid-height be broader in the presence of copolymer is indicative of a better PLA/Mg adhesion. Subsequently, the bioactivity of PLA/Mg composites was studied in the SBF during three months. The analyses showed that the addition of copolymer accelerated the formation of $Mg(OH)_2$ on the surface as a first step. Then, a formation of the hydroxyapatite in bulk was revealed, which could induce a good osteoconductivity. Finally, an increase in the protein adsorption was proved by the addition of the copolymer as expected, but a slight decrease in the cell adhesion was observed.
The PEO-*b*-PLLA block copolymer was used as a compatibilizing agent and, as an original approach, to modulate the bioactivity of PLA/Mg composites. To fulfill the target of this study, a compromise must be reached between the use of copolymer which could both improve the PLA/Mg interface and the bioactivity behaviour, while affecting the mechanical performances of the composites.

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ATMOSPHERIC PLASMA: A SIMPLE WAY TO IMPROVE THE INTERFACE BETWEEN POLYSACCHARIDES AND POLYESTERS

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Due to the exponential increase of studies in the area of biomaterials, surface modification became a target in order to improve performances of new devices for wound healing and replacement of living tissues [1]. Indeed, improved performances (mechanical and biological) often pass through imparting compatibility between the surface of synthetic polymers (mainly hydrophobic) and natural polymers (hydrophilic). With this respect, numerous studies were done on the grafting or non-covalent deposition of natural polymers on the surface of synthetic ones. The difficulty is due to the incompatibility of the surface chemistry between both types of polymers. To overcome this problem, some authors use plasma technology – a method quite poorly reported despite its obvious advantages (low time and energy consumption, excellent reproducibility, ease of industrialization, etc.) [2–5].

Taking advantage, the present work uses an atmospheric plasma torch to improve the (predominantly non-covalent) deposition of chitosan (natural polysaccharide), for its antimicrobial and cell anchoring sites, on a poly (L-lactide) (PLA, synthetic polyester) surface for its mechanical and biodegradable properties. First, PLA plates were surface-activated by atmospheric plasma treatment. Several parameters, such as treatment time, torch distance and power have been varied for process optimization. At the optimal conditions, the contact angle (CA) of the PLA plate decreased by 20° because of the hydrophilic function introduced by the plasma. The morphology of the surface after plasma activation observed by atom force microscopy (AFM) showed a structured (nodular) morphology. On this activated surface, a chitosan solution was deposited by spin coating and a smooth coating formation was observed after solvent evaporation. The successfulness of the deposition process of the chitosan was confirmed by the presence of amide and amine bands in the FTIR and X-ray photoelectron spectrometry (XPS) spectra, further CA decrease and smoother, non-nodular morphology shown by AFM. Thus, this new plasma process seems to be an easy and versatile way to activate the surface of fibers to graft a bioactive layer.

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1ST ON-LINE WORKSHOP
SURFACE **E**NGINEERING FOR **B**IOMEDICAL **A**PPLICATIONS
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A FEMTOSECOND LASER-BASED STRATEGY TO MODULATE THE RACE FOR THE SURFACE: MICRO/NANOSTRUCTURED CERAMICS SURFACES TO IMPROVE OSTEOGENIC DIFFERENTIATION AND DIMINISH BACTERIAL ADHESION

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Surface topography at the micro and nanoscale levels has shown to induce a positive effect on bone regeneration by enhancing cell adhesion and proliferation, up-regulating specific cytoskeletal and extracellular matrix proteins, and improving osteogenic differentiation. However, implants with increased surface roughness are usually associated with increased bacterial adhesion.

In this work, a femtosecond laser ablation technique was used to structure the surface of Alumina toughened Zirconia (ATZ) at the micro and nanoscale levels. The main goal was to enhance the surface functionality of the ceramic, for improved osteogenic differentiation of hMSCs while decreasing bacterial adhesion. Femtosecond laser is a versatile tool for surface modification with high reproducibility, commonly used on metals and that has been increasingly applied on ceramic materials. Materials characterization was performed before and after laser treatment using SEM/EDS, FTIR, XRD and contact angle measurements. The influence of the topographical stimuli on the osteogenic differentiation of hMSCs and adhesion of Staphylococcus aureus was assessed.

Microtopography was successfully developed with overlapping of high-frequency laser induced periodic surfaces structures (LIPSS), that nanotextured the ceramic surface in a perpendicular direction. No changes were observed in the chemical composition of the materials due to laser treatment.

hMSCs proliferation increased with the time of culture and cells adhesion and proliferation was modulated mostly by the microtopography. The laser treated ATZ displayed a higher expression of osteogenic-related markers and mineralized extracellular matrix, after 21 days of culture. Regarding the bacterial adhesion, CFU counts revealed that there was a significantly lower S. aureus adhesion on the laser treated surface, when compared to the untreated ATZ. The results were confirmed by CSLM and SEM microscopy.

Surface modification by femtosecond laser constitutes a simple, non-chemical, single-step process to create precise and reproducible micro- and nano- textures on ATZ. These ceramic biomaterials with improved surface functionality may be used in dentistry and load-bearing applications, to enhance osteogenic response and decrease infections risks.

STRUCTURE AND BIOCOMPATIBILITY OF PVD DEPOSITED TIN/TiO_2 COATINGS ON ELECTRON BEAM TREATED TI6AL4V ALLOY

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Due to their exceptional properties, titanium and its titanium alloys are proved to be indispensable materials in biomedicine. They are considered the most biocompatible among all metals and alloys due to their high corrosion resistance in various body fluids, high fatigue limit, remarkable specific strength, and bio-inertness. Titanium can naturally form a dense passivating oxide layer on its surface that is insoluble and chemically impermeable, protecting the metal from the surrounding environment. Ti6Al4V is commonly used in the orthopedic application for internal fixation prosthesis, screws, and plates, artificial bones, and joints. However, the poor tribological performance characterized by severe adhesive wear and the high friction coefficient is the main limitation of titanium alloys [1].

For the reasons mentioned above, the research on titanium alloys for medical application is concentrated on surface treatments to improve the poor tribological properties and enhance the biocompatibility of the Ti6Al4V alloy. The development of suitable surface treatments has to effectively increase the near-surface strength, hardness, wear-resistance, thus reducing the friction coefficient together with avoiding possible corrosion problems. To accelerate the osseous integration of hard implants and enhance the bone-implant contact, a new generation of prothesis with modified surfaces and features at microtopographic and superimposed second nanotopographic level of roughness are widely studied. The aim is to shorten the healing time and to improve the anchorage of the implant in the fractured or ill area.

To enhance the performance of titanium alloys, different mechanical, chemical, electrochemical, or physical surface methods were found to be able to achieve all these objectives. A modification of the physical, mechanical properties and roughness of the near-surface area of solid can be obtained after electron beam treatment (EBT). In that way, wavy microtopography and increased surface hardness were successfully introduced on the Ti6Al4V surface. The samples thus treated were subjected to PVD coating deposition that additionally improve the surface topographic and chemical characteristics while increasing their durability and corrosion resistance. PVD deposited TiN films are among the first patented coatings for biomedical applications [2]. However, PVD deposited hard and wear-resistant TiN exhibits less pronounced biocompatibility to form a direct bond with bone as compared with TiO_2 [3]. Because of their innate high hardness, toughness, chemical stability, and biocompatible nature, bi-layered nanostructured coatings of TiN/TiO_2 have been deposited by cathodic arc evaporation (CAE), and magnetron sputtering (MS) on the surface of the electron beam treated Ti6Al4V alloy. Except for adequate interaction between implant material and surrounding tissue, the surface TiO_2 ensures antibacterial properties by its photocatalytic activity [4].

By varying the scanning frequency (f) of the electron beam from 500 (AR500) up to 850 (AR850) Hz and keeping the other technological parameters (speed of the sample motion (ν) - 2 cm/s; electron beam current – I = 30 mA, accelerated voltage – U = 52 kV linear manner of scanning) constant, after the EBT the surface hardness and roughness were increased. As opposed to the as-received (AR) specimen (Ra = 0.14 μm and 323 HV0.2), the highest values of surface roughness (R_a = 1.97 μm) and average hardness (405.4 HV0.2) were measured for AR500 samples followed by AR850 where the hardness was almost 387.5 HV0.2 whereas the R_a was equal to 1.58 μm. On the wavy re-melted and a rapidly cooled top surface of the treated samples, round-shaped grains with precipitated martensite laths in them were seen. The average distance between the grooves was found to be 42.67±1.58 μm and 32.34±0.82 μm for the AR850 and AR500 samples, respectively. Nonetheless, the average maximum peak-to-valley (Rz) values were equal to 5.84±0.17 μm and 7.35±0.21 μm, respectively.

After the deposition of TiN/TiO_2 coatings, substantial differences between the CAE and glow discharge oxidized and MS films were observed. White the 1 μm thick reactive magnetron sputtered TiN/TiO_2 coatings almost entirely copied the surface characteristics of the substrates, the 3.7 μm thick CAE films were found to be less smooth because of the presence of "microparticles" protruding from the nitride sub-layer and covered with oxide crystals. These droplets with conical morphology were homogeneously distributed in and over the coating. Moreover, in contrast to the mono-phase polycrystalline TiN in both types of coatings, the phase composition of MS and glow-discharge deposited TiO_2 layers was found to be different. The MS oxide had a monophasic structure of anatase while the glow-discharge deposited TiO_2 showed biphasic rutile and anatase composition. The nanohardness of the MS coating on the polished substrate reached about 7.1 GPa, while that of the CAE films was equal to 5.3 GPa.

When immersed in simulated body fluid (SBF, pH 7.4) at 37 °C for 7, both CAE and MS TiN/TiO_2 coating demonstrated the ability to nucleate apatite directly on TiO_2 layer because of the increased number of OH- groups at the surface. By further increasing immersion time up to 14 days, apatite nuclei became larger and more agglomerated. The pH changes of SBF in the time indicated a slight decrease during the first days of immersion because of the de-protonation of acidic hydroxides. After that, pH values slowly increased along with the immersion period. Both CAE and MS coated samples showed similar trends in pH variations. Simultaneously, after 7 and 14 days of immersion in SBF, corrosion and steady-state potentials of both tested coatings shifted towards nobler direction indicating that the samples became less prone to corrosion. The obtained j_{corr} for both CAE and MS coated samples showed extremely low values after 7 and 14 days of immersion in SBF while polarization resistance was significantly higher. These results confirm the biocompatibility and stability of tested coatings in a synthetic solution that has ion concentrations similar to human plasma.

Since the surface topography and chemistry play a critical role in the interaction of the artificial graft with the adjacent tissue, the cell adhesion, viability, and bone mineralization of MG-63 osteoblast cells were compared using adhesion and MTT assays, and fluorescent microscopy. Within the first 6 hours, osteoblast cells attached faster on the CAE coated samples than on the bare AR alloy and MS coatings except for the polished MS coated sample, where the cell attachment was comparable with that of the CAE coating. The coated substrates indicated superior attachment of MG63 cells as compared to that on Ti6Al4V plates, demonstrating that their nano-topography provides a larger contact area at the medium-sample interface. The results of cell viability indicated a better growth of MG63 cells on TiN/TiO_2 coatings, especially on CAE films, when compared to bare alloys except for the MS coated

AR500 sample where the cell viability was decreased. It follows that the CAE and glow discharge oxidized films provided a better environment for cell growth as opposed to the bare and MS coated samples. After 31 days of incubation, the mineralization process in MG63 cells cultured on both coated and uncoated samples showed mineralized areas of differentiation-induced calcium nodules in all tested surfaces with different quantities and locations. Among all bare and MS coated alloys, the mineralized areas at AR850 were the lowest, while within CAE coated samples, those on the AR500 specimens were the most highly mineralized. The obtained results confirm that the combination of EBT microtopographic and superimposed nanotopographic level of roughness and chemical composition of TiN/TiO_2 CAE coating promoted cell adhesion without inhibiting cell viability better than uncoated and MS coated samples and demonstrated more significant bone mineralization potential of MG63.

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THE ALTERED BEHAVIOR OF MESENCHYMAL STEM CELLS ON 2D COLLAGEN MATRICES IN OXIDATIVE ENVIRONMENT

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Mesenchymal stem cells (MSCs) are strongly involved in the process of extracellular matrix (ECM) remodeling due to their important role in the regenerative cell-based therapies. Abnormal ECM remodeling upon oxidative stress might dysregulates balance between the formation and degradation of ECM leading to various pathological conditions.

Here we aimed to establish the biological response of human adipose derived MSC (ADMSCs) adhering onto native and pre-oxidized collagen isolated from mouse tail tendon with an attempt to mimic the conditions of oxidative stress *in vivo*.

Collagen Type I isolated from mouse tail tendon (MTC) was labelled with FITC. This labeled native collagen was either oxidized or not using previously established Fe2+/H2O2 protocol. The effect of collagen oxidation on the ADMSCs remodeling activity was evaluated morphologically, also in the presence or absence of antioxidant epigalocatechin gallate (EGCG).

Morphological studies demonstrate that MTC undergoes significant remodeling by human ADMSC involving a mechanical translocation of adsorbed protein and a tendency for fibril-like organization. The formation of dark streaks of collagen removal reflecting the cellular proteolytic activity was also observed. An important observation is that the ADMSC remodeling is inhibited on oxidized collagen samples. Though some less organized fibers still exist the proteolytic activity is absent. Appearance of small collagen aggregates is the most common morphological finding. These aggregates accumulates mainly around the cells, particularly in the EGCG samples**,** suggesting the involvement of ADMSCs in their formation.

The oxidative environment affects dramatically the stem cell-dependent collagen remodeling leading to protein fragmentation and block the pericellular proteolytic activity. A novel observation is that the antioxidant EGCG may potentiate the collagen fragmentation.

ELECTRON-BEAM SURFACE ALLOYING OF TI SUBSTRATE WITH TA FILMS

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Titanium is widely used in the field of modern medicine, due to of its excellent resistance to corrosion, high stiffness and toughness and remarkable strength-to-weight ratio. It is known that the incorporation of Ta and formation of Ti-Ta alloys is promising because they exhibit high temperature shape memory effect and hence at human temperature, they are in highly elastic states comparable to those of muscle tissues. Additionally, it is known that doping of Ti alloys with Ta leads to an increase in the corrosion resistance and biocompatibility [1]

In the present study, Ti-Ta surface alloys are formed by means of selective electron beam alloying, as on commercially pure Ti substrate, Ta film with thickness of 1 μm was deposited by DC (direct current) magnetron sputtering. The obtained samples were then electron beam surface alloyed. The alloying process was carried out by oscillating electron beam with circular trajectory. During the alloying process, the accelerating voltage was 52 kV, the electron beam current was from 15mA to 35 mA, the speed of the specimen motion was 5 mm/s, the electron beam scanning frequency was 200 Hz, the diameter of the electron beam was 0.5 mm. The microstructure of the obtained surface alloys was investigated by Scanning Electron Microscopy (SEM), the chemical composition was studied by Energy Dispersive X-ray Spectroscopy (EDX). X-ray diffraction (XRD) methods were used to determine the crystallographic structure. The mechanical properties were also characterized and the results are discussed with respect to the applied technological conditions and the structure of the samples.

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ROUGHNESS AND MECHANICAL PROPERTIES OF ELECTRON BEAM SURFACE MODIFIED AND TIN/TiO_2 COATED TI6AL4V ALLOY FOR BIOMEDICAL APPLICATION

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Various surface modification techniques such as mechanical, chemical treatments or deposition of coatings have been applied for enhancing the mechanical properties and osseointegration of titanium alloys. In this study, we use PVD deposition of TiN/TiO_2 coating on polished and electron beam treated Ti6Al4V alloy to compare the change in roughness and surface mechanical properties of the coated systems. The films were characterized by scanning electron microscopy, atomic force microscopy, nanohardness measurements, and ball-on-wear tests. After the electron beam treatment (EBT), the average surface hardness and roughness increased from 323 HV0.2 and 0.14 μm up to almost 387.5 HV0.2 and 1.58 μm, respectively. After coating with 3.7 μm thick TiN/TiO_2, the average S_a roughness and nanohardness of the film on the polished substrate reached 0.87 μm and 13.05 GPa while that on the EBT was equal to 1.57 μm and 8.66 GPa, respectively. The drop in the hardness value could be attributed to the higher surface roughness values [1] because the flat samples exhibit better surface properties. However, the comparison of the evolution of coefficients of friction (COF) of the substrates and the coated specimens indicated a decrease in COF at about 0.18 for the coated EBT alloy as compared with the polished and EBT substrates which average COF were equal to 0.45 and 0.38, respectively. High COF (0.67) was registered for the coated as-received alloy because of the gradual and complete worn off of the film during the test. It follows that the combination of initial EBT of Ti6Al4V alloy together with the deposition of PVD TiN/TiO_2 coating could substantially improve the roughness and tribological outcomes such as friction of the surface. However, a further tribological test in an environment that replicates biological rights are needed to confirm the acceptability of these findings.

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SURFACE MODIFICATION OF Co-Cr-Mo ALLOYS BY ELECTRON BEAM TREATMENT

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The Co-Cr-Mo alloys are widely used in the field of modern medicine and have many biomedical applications. However, the biological corrosion leads to a separation of metallic ions which results in adverse reactions and implant failure. This depends mostly on the surface properties of the materials and can be overcome by an appropriate technology for surface manufacturing. Up to now, electron-beam surface treatment of materials receives a lot of attention due to the possibility of precise control of the technological conditions. This technique has a major advantage in comparison with the other methods, namely beam deflection. This allows a realization of scanning with oscillating beam with different trajectories, such as linear, circular, sinusoidal, etc., which leads to different cooling rate and, therefore, different surface structure and properties.

In this study we present results of investigation of the influence of different electron beam trajectories on the surface structure and properties of Co-Cr-Mo alloy. During the experiments, the accelerating voltage (U) was 55 kV, the speed of the sample motion (V) was from 0.5 to 5 cm/s, the electron beam current (I_b) was from 10 to 20 mA, the electron beam scanning frequency (f) was from 1 to 10 kHz, the diameter of the beam was 0.5 mm. The structure was studied by X-ray diffraction (XRD), scanning electron microscopy (SEM) and energy dispersive x-ray spectroscopy (EDS). The mechanical properties were investigated by nanoindentation test. The results obtained are expected to add knowledge and extend the understanding of the processes of the formation of modern implant materials.

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BIOGENIC FERROXIDES FOR APPLICATION IN ELECTRONICS, BIOMEDICINE AND BIOTECHNOLOGY DERIVED FROM *LEPTOTHRIX* BACTERIA

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The present work is focused on studying by-products derived from the metabolism of bacteria of the *Leptothrix* genus, which are one of the first described microorganisms associated with the iron cycle in nature. The products of their metabolism are nanostructured biogenic iron oxides in the form of precipitating powders and sheath structures. The sheath structures can be considered as an organic matrix in which inorganic crystallites are discretely dispersed. We used X-ray diffraction, magnetic measurements (PPMS), light microscopy and scanning electron microscopy (SEM) to characterize biogenic products formed in an SIGP medium under laboratory conditions. The studies showed a lack of significant differences between the naturally obtained and the artificially synthesized biogenic sheaths, i.e., an adequate laboratory technological process has been developed. From the point of view of nanoelectronics application, these biogenic by-products are unique because they are biocompatible, have specific electromagnetic properties and can be potential candidates for various applications in biomedicine and electronics.

INVESTIGATION OF THE DEVIATION DURING THE INFORMATION TRANSFER FROM THE PROSTHETIC FIELD TO THE LABORATORY SCANNERS

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The aim of this in research study is to investigate the deviations during the first stage of the information transfer from the prosthetic field to the dental laboratory scanners for CAD/CAM technology.

The first upper left molar on artificial acrylic resin dental model was prepared for full metal-ceramic crown according standard guidelines for step cut. With high precise industrial scanner, the prepared prosthetic field was scanned with 1 mm ruby ball stylus and a digital copy was made. With standard tray, from the acrylic model, two-phase two-stage silicon (A-silicone Zhermack hydrorise putty and light body) impression is taken following recommended technology of manufacturer. The impression is powdered with dental stone type IV and a stone model is created 1h after impression, similar to time in dental practice. Using the same measurement methods, the stone model's surfaces were digitised. Both digital models were compared using acrylic resin dental model as reference. Results about the deviation during the transfer of information between the prosthetic field and the stone model are first level of inaccuracy in CAD/CAM technology processing.

The difference of dimensional alteration for each dimension, according to reference and stone model is not more than 20μm in shrinkage or expansion direction. Thus the widely used approach for information transfer with stone models from the prosthetic field to laboratory scanner for CAD/CAM technology could be considered as relevant with around ±10μm inaccuracy.

If the silicon impression is performed carefully the stone models are enough accurate representations of the prosthetic field and their digitalisation in dental laboratory is reliable starting point for CAD/CAM technology.

EFFECT OF DEPOSITION PARAMETERS ON THE STRUCTURAL AND MECHANICAL STABILITY OF Ta-BASED COATINGS DEPOSITED ON POLYMERS FOR BIOMEDICAL APPLICATIONS

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Ta-based materials and coatings are excellent endoprosthetic biomaterials for use in trauma and cardio-vascular surgery. Tantalum was selected as an alternative to other commonly used metallic material for orthopedic implant applications. The biocompatibility and antibacterial properties of tantalum coatings were previously reported. Tantalum-based thin films have antimicrobial potential against several microorganisms, such as Staphylococcus aureus, Staphylococcus epidermidis. Polymer materials are used in a wide range of medical devices. Non degradable polymers are applied as bearing surfaces in hip implants; vascular grafts or catheters; and ocular implants due to structural stability and biocompatibility. Hernioplasty mesh prosthesis are commonly used for substitution of aponeurotic and muscle tissue. Currently used polypropylene meshes induce severe chronic inflammatory reactions over the long term period. These processes lead to scarring and have a negative effect on the connective tissue and abdominal walls. Moreover, the influence on the apoptosis rates due to the polymers destruction, their degradation products, and further cancerogenic potential based on apoptosis rates were previously reported. Bio functionality of polymer materials can be improved by surface modifications.

The aim of the present study was the investigation of an effect of process paremeters on the structural and mechanical stability of Ta, Ta_2O_5 and TaN films deposited on polypropylene substrates and mesh. The process of Ta, Ta_2O_5 and TaN coatings deposition by magnetron sputtering method was performed. The magnetron discharge power was 4-5 kW. ICP source for oxygen activation with RF power up to 1 kW was applied. The effect of deposition process parameters such as sourse power, pressure, and distance to target on the structural and mechanical properties of Ta-based coatings was analized.

The coatings thickness and adhesion properties were evaluated by standard methods. The surface morphology and topography were observed by electron scanning microscope JSM-7100F (SEM, JEOL, Japan). The elemental distribution and chemical composition of the coatings were analyzed by energy dispersive X-ray spectroscopy (Oxford Link ISIS 300).. X-ray photoelectron spectroscopy was carried out using ESCALAB MkII (VG Scientific, UK). Mechanical tests of Ta-based coated samples for evaluation of Vickers hardness parameters and elastic modulus were performed on a Nano Indenter G200 (KLA, USA).

At present study, Ta-based films favourable properties for potential prosthetic applications in hernia surgery are proposed. Results demonstrate the strong influence of the deposition parameters on the composition and structural properties of Ta-based coatings. The coatings deposition process resulted in the structural and mechanical stability of polymer materials for biomedical applications.

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THE DIGITAL AXIOGRAPH – A NOVEL TOOL IN BRUXISM PREVENTION

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Bruxism in dentistry is considered an oral parafunction of involuntary grinding and clenching of the teeth. Occlusal splints are the most common method of prevention of bruxism and its consequences. That very prevention of the parafunction is reported to be the proven way of treatment. Splint application significantly reduces the pathologies of the dental and periodontal structures. The parafunction itself is a controversial topic and therefore constantly investigated. The digital (optical) axiograph is an innovative facial bow that allows digital recording of individual data on the movements of the lower jaw. This helps the clinician to better analyze the disturbances that have occurred as a result of occlusion and articulation problems, and to determine the correct position of the mandible. The technique of electronic axiography allows the registration of all parameters necessary for the manufacture of occlusal splints and orthopedic structures. Digital axiography is an applicable, promising technology with proven accuracy, comparable to conventional axiographs and superior to them with better interpretation of the data obtained.

The purpose of the study is to develop a protocol of planning and producing a preventive occlusal splint for bruxism management by means of digital axiography.

A 42 year old patient is clinically and objectively diagnosed with sleep bruxism, using a standard questionary, oral examination and a BiteStrip® disposable miniature electromyography device. Patient's data is obtained by alginate impressions of upper and lower jaw. Plaster impressions were poured and scanned with Ceramill Map 600 optical desktop scanner. Data of mandible position and movement trajectories were registered with Dentograf digital axiograph and along with CBCT and scan data was uploaded and processed in Dentograph's software. After profound data analysis, an occlusal splint is designed in exocad software and 3D printed with a Form 2 three dimensional printer using Dental LT Clear photopolymer resin.

Profoundly clarifying and simplifying the steps of a new, predictable and precise, fully digital approach for prevention of bruxism from the initial diagnosis to the manufacture of splints and case tracking.

Successful development and implementation of an effective, fully digital approach in the diagnosis of bruxism, which minimizes the error possibility, to facilitate and classify the steps in the prevention of bruxism. Together with the method of 3D printing occlusal splints, to fill the missing link for a complex, precise method for successfully dealing with such cases in everyday practice.

ALL-OPTICALLY CONTROLLED DENSITY OF ALKALI ATOM VAPORS FOR BIOMAGNETIC SENSING APPLICATIONS

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Biomagnetic sensing is a powerful non-invasive method to measure the magnetic fields induced by the currents of living organisms such as magnetocardiogram (MCG), which detect magnetic field from the heart, magnetomyogram (MMG) - from the muscles, and magnetoencephalogram (MEG) - from the brain. The main concept behind the biomagnetism is based on ions which move between the living cells and induce extremely small magnetic fields compared to the magnetic interference from the surrounding environment. In order to detect such small biomagnetic signals very sensitive magnetometers are required [1,2].

Optically Pumped Magnetometers (OPM) have the sensitivity of superconducting quantum interference device (SQUID), which were leading in the field of extremely low magnetic sensors. Unlike SQUID, OPM does not require cryogenic cooling and OPM can measure magnetic fields without additional calibration [3]. OPM are based on single frequency tunable diode lasers which are in resonance with alkali atom vapors (usually Rb, Cs or K) confined in very small optical vacuum cells (a few mm long) to achieve high spatial resolution [4]. To improve the sensitivity of OPM magnetometers, spin-relaxation of atoms with the cell's walls is reduced by anti-relaxation coatings on the inner surface and/or buffer gas. Another way to improve the sensitivity is to increase the density by heating [3].

In this communication we report our investigations on all-optical control of atomic density in optical cells for application in magnetometry. A special system for homogeneous illumination of the cell walls is used to increase the atomic density, to avoid the deposition of alkali atoms on the cell walls and reduce deterioration of the coating [5].

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GENETIC LANSCAPE OF PRIMARY LUNG ADENOCARCINOMA - SINGLE INSTITUTION EXPERIENCE

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Cancer is now known as a disease of genomic alterations. The role of genetics in lung cancer development, a leading cause of cancer death worldwide, is becoming even more important in the last decade. Mutational analysis and genomics profiling have advanced the field of lung cancer research, emphasizing the critical role of some driver mutations in disease occurrence, progression and treatment response. Molecular testing has become an essential part of oncology, as the number of molecular biomarkers and viable therapeutic targets for the treatment of patients with non-small cell lung cancer (NSCLC) continues to expand. NSCLC (comprising 80% of all lung cancer cases) remains one of the major public health problems, with very poor prognosis, treatment response rate and a 5-year-survival rate of less than 15%. The discovery of numerous specific molecular alterations in the last decade has led to the tremendous changes in the treatment strategy, moving from standard therapies to novel personalized approaches including targeted therapies. The main goal and challenge of the personalized medicine is to improve the diagnosis and efficacy of therapy, thus promoting longer survival of patients and better quality of life. Critical point in achieving that aim is the establishment of a precise diagnostic tool which would enable more efficient and prompt identification of the genetic background of the tumor.

The aims of our pilot study is to investigate the genetic profile of primary lung adenocarcinoma in the Bulgarian population and to compare the accuracy and practical value of two diagnostic approaches with clinical significance for the routine testing – PCR-based detection through rt-mPCR (real-time, multiplex polymerase chain reaction) and NGS (next generation sequencing).

DNA-samples of 10 patients (3 male, 7 female) with stage IIIB-IV lung adenocarcinoma was extracted from formalin-fixed, paraffin-embedded (FFPE) tumor tissue. Initial testing was performed in search of known EGFR-driver mutations by rt-mPCR. Subsequently, a NGS-analysis with TruSight Cancer Panel, Illumina® (94 genes and 284 SNPs) and/or ArcherDx (Archer® VariantPlex Solid Tumor panel) was performed on Illumina MiSeq device. Bioinformatical pipeline included BaseSpace (for alignment and variant calling) and VariantStudio analysis. The effect of rare (<3% global frequency) missense variants was predicted using RadialSVM and LR scores. Variants were classified into 3 groups – pathogenic mutations associated with cancer development (driver mutations), probably pathogenic variants that might contribute to disease progression and prognosis, and variants of unknown clinical significance.

At least one clear-cut driver mutation was identified in each patient sample. As expected, the most prevalent genetic alteration in lung adenocarcinoma were those in *EGFR*, particularly c.2572T>C, (p.Leu858Arg) and EGFR (c.2369C>T, p.T790M) in patients with secondary TKI-inhibitor-resistance, as well as in *KRAS* (c.32G>T, p.G12C). A novel, undescribed variant in *EGFR* (c.2506_2507insAA, p.Arg836GlnfsTer11), was identified in one sample. Given its biological effect – frame-shift with occurrence of preterminal stop-codon, we can hypothesize its etiological importance. However, further

analysis is necessary, in order to understand the potential meaning in TKI-therapy response. Results from NGS confirmed data from initial PCR-based testing and revealed new variants with potential clinical significance.

The great heterogeneity in all patients highlights the importance of the comprehensive, high-throughput approach in NSCLC genome testing for identifying targets with clinical significance and of the personalized approach. Given the small sample size and the abundance of biomarkers with clinical relevance in NSCLG, we believe that NGS must be routinely integrated into practice as a time- and tissue-sparing method compared to PCR. Furthermore, establishing a centralized database of driver and other genetic alterations in the Bulgarian population is essential for the future research, new target identification and treatment options development.

ATMOSPHERIC PARTICULATE MATTER POLLUTION OVER RESIDENTIAL URBAN AREAS DURING COVID-19 QUARANTINE

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This study of Particulate matter (PM) pollution was conducted during the quarantine period COVID-19 in the residential areas of the capital of the Republic of Bulgaria - Sofia (Mladost and the lower part of Lozenets), which have more than 160 thousand inhabitants. At present, the control of air pollution is concentrated mainly on the content of PM2.5 μm and PM10 μm, respectively. There are stationary licensed stations of the Ministry of Environment and Water and the civil network in the study area. The methodology subject of the present study is based on lidar monitoring of the ground layer of the atmosphere, as the lidar scans an area with a radius of up to 20 km and can give the current state of the PM in the atmosphere.

An important element of the methodology, which is the focus of this study, is the sampling. For this purpose, *in situ* in areas with increased air pollution, fixed on the city plan during the lidar monitoring with portable instruments, sampling is performed. For this purpose are used: Absorber with filters, where PM was fixed, Cascade impactor, which separates the particles by size for examination of the microbial content; Electronic sensors for determining the mass concentration of PM2.5 and PM10. After sampling, the collected PMs were examined by a set of physical methods (XRD, SEM, EDAX, MëS and PCR tests for microbial contamination). A wide range of data was obtained on the crystal chemical composition of PM, morphology, nature of the surface and interior of the particles, their microbiota and elemental composition. Studies show a wide variety of particles, which strongly depends on seasonal characteristics (flowering, moisture, etc.). A common feature is that most of them are aggregates of PM, where in a peculiar way they combine inorganically and organically. The study focuses on the content of nanoscale PMs in the atmosphere that are traffic-related and that are particularly dangerous to human health.

Key words: lidar monitoring, particulate matter (PM) in atmospheric bioaerosol, microbiological investigation, COVID-19 quarantine period

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11 November 2020, Bulgaria

3D BIOPRINTINNG OF VASCULARISED BONE TISSUE: CURRENT ADVANCES AND CHALLENGES

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Vascularisation in bone tissue facilitates the distribution of nutrients and oxygen, as well as the elimination of waste products. Fabrication of tissue-engineered bone constructs which possess functional vascular networks enables recapitulation of the human bone tissue microenvironment in vitro. A number of different approaches have been applied to generate biomimetic vascularised tissue-engineered bone constructs. Traditional approaches involve cell seeding into two-dimensional (2D) scaffolds but those are unable to precisely recreate the spatial architecture and the encapsulated distribution of bone tissue cells. In recent years, the pioneering field of three-dimensional (3D) bioprinting has been an emerging and accurate tool to fabricate vascularised tissue-engineered bone constructs layer by layer. 3D bioprinting technology enables the fabrication of 3D vascularised models with precisely controlled tissue architecture and cell distribution, which holds great potential for drug screening and disease modeling. This review discusses the role of novel 3D bioprinting approaches in the generation of vascularised tissue-engineered bone constructs as well as some of the major challenges of fabricating functional vascular networks.

Keywords: 3D bioprinting, tissue engineering, vascularisation, bone regeneration bioinks

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STEREOTACTIC VACUUM ASSISTED BIOPSY OF THE BREAST - CURRENT INDICATIONS AND TECHNICAL CHALLENGES

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For early screening for breast cancer mammography has been used. Early detection of suspicious malignant lesion required adequate biopsy tool. Stereotactic vacuum assisted biopsy (SVAB) is a minimally invasive procedure in which a sample of breast tissue is removed for examination. SVAB is a less invasive alternative to wire guided excision biopsy in nonpalpable breast lesions which are not seen on ultrasound.

To present the current indications for SVAB in the breast lesions diagnosis and to demonstrate some technical challenges in our initial experience. To the best of our knowledge, this is the first experience with SVAB in Bulgaria.

From March 2020 to October 2020 stereotactic vacuum assisted biopsy was performed in eleven patients with suspicious breast malignancy lesions. Siemens Stereotactic Breast System and Hologic Brevera Breast Biopsy System were used. All procedures were done under local anesthesia. Technical difficulties, intervention time, complications, and accuracy were evaluated. A nonsystematic literature review for the indications and contraindications of SVAB in the breast lesions was performed using the databases of PubMed, Scopus, EMBASE and Cochrane Library. This work was supported by the European Regional Development Fund through the Operational Programme “Science Education for Smart growth” under contract №BG05M20P001-1.002-0010-C01(2018-2023).

All authors have described for absolute indications of SVAB: grouped calcifications, non-palpable lesions of the breast, architectural distortion, focal palpable or nonpalpable mass after negative core needle biopsy. Contraindications described in the literature for SVAB: negative mammograms lesions, previous excision, abnormal coagulation, claustrophobia, and an impossibility for breast fixation on the table.

In our cohort first three cases were done under proctoring by an experienced radiology team. An interdisciplinary team between surgeons and radiologist was organized. Radiologist is responsible for the stereotactic system and surgeons - for the skin incision and vacuum biopsy device insertion. Medial intervention time was 47 minutes. In the last 3 cases, it was improved to 33 minutes. In one patient postinterventional hematoma was diagnosed. In 5 of 11 cases, breast cancer was proven. Most difficult localization of the target zone was the upper quadrants near the chest wall.

SVAB is a safe minimally invasive procedure with less trauma and smaller scar than other procedures for nonpalpable breast lesions which are not seen on ultrasound. The learning curve needs to be considered. An interdisciplinary team between surgeons and radiologists could decrease the rate of technical difficulties. Since these are the preliminary results more data is to be collected.

CLINICAL APPLICATION OF NEXT GENERATION SEQUENCING TECHNOLOGY

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Nowadays, Next generation sequencing (NGS) is a valuable tool mainly for research settings with a potential to be used in clinical laboratories for genetic diagnosis. We present the application of NGS technologies within the framework of project "CENTRE OF COMPETENCE IN PERSONALIZED MEDICINE, 3D AND TELEMEDICINE, ROBOTIC-ASSISTED AND MINIMALLY INVASIVE SURGERY".

The first research field of the project is directed towards inherited disorders and the potential of pre reproductive carrier screening to reduce their incidence. Carrier screening (CS) is traditionally targeted towards Mendelian disorders, mainly autosomal recessive or X-linked. The application of NGS technology for screening purposes allows fast and efficient testing of many genes and thousands of mutations simultaneously. In couples with detected gene mutations, genetic counseling provides accurate information about the risk of having an affected child and gives opportunity to make informed reproductive decision and applying of prenatal /pre implantation diagnosis.

The second research field is Oncogenetics, particularly breast (BC) and ovarian cancer (OC). NGS technologies improve ability to identify (by germ line test) individuals at risk for BC and OC as well as to clarify cancer diagnosis, guide treatment and modify prognosis, based on tumor genetic profiling. The comparative analysis of germ line and somatic genetic alterations could be a useful tool to move the treatment recommendations in affected individuals from a "one-size-fits-all" to a tumor-specific, precision cancer therapy.

NGS technology facilitates the accurate molecular diagnosis of genetically heterogeneous inherited disorders, identification of carriers of genetic defects associated with risk for reproductive failure/affected children or cancer predisposition.

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