Втора научна сесия

„Биомедицина и качество на живот“

за млади учени, докторанти и постдокторанти

в чест на 150-та годишнина на Българска академия на науките

СБОРНИК С АБСТРАКТИ

Институт по биофизика
и биомедицинско инженерство – БАН

4 – 5 ноември 2019

Втора научна сесия „Биомедицина и качество на живот“ 2019

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Session „Biomaterials, Cell and Tissue Engineering"

Biophysical methods for fast determination of lung maturity in newborns

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Abstract: The aim of our study is to establish new approaches for lung maturity assessment in risk prematurely born children with extremely low weight, born after *in vitro* fertilization and multiple pregnancy on the basis of gastric aspirates (GA) as non-invasive and painless clinical samples collection. In addition of clinical tests, till now lung maturity was determined by *in vitro* biochemical and biophysical analyzes of maternal amniotic fluids, tracheal or nasopharyngeal aspirates of the newborn children, etc. Procedures of clinical samples collection are invasive, traumatic, painful and insufficiently informative. Therefore, it is important to search new, fast, non-invasive methods with greater sensitivity and specificity in order to undertake timely and adequate treatment of at-risk infants. Gastric aspirates is an appropriate clinical sample, which is collected by quick, easy and non-invasive procedure in the first minutes after birth. Because the fetus swallows both amniotic fluid and lung fluid, secreted during fetal development, GA contains components of amniotic fluid and lung secretions as well as secretions from the stomach and esophagus. The study included 67 infants divided into two groups: 23 prematurely born infants developing clinical signs of NRDS and treated by assisted ventilation and exogenous surfactant, and 44 full-term healthy babies (control group). The assessment of surfactant maturity in GAs was realized for the first time by the combination of two modern techniques, axisymmetric drop shape analysis (ADSA) of a pendant drop and Brewster angle microscopy (BAM).

We can summarize our results in the following conclusions:

- Minimum surface tension values, obtained by ADSA analysis, are statistically and significantly lower in the control group of healthy infants compared to high-risk infants. This parameter correlates with lung maturity and it is an appropriate marker for assessment of lung maturity.
- BAM images of the GAs monolayers from the control group showed better structured thick and dense surface films with contrast brighter domains, than those of the risk group that were extremely thin as a result of the lack of surface active component.
- The gastric aspirates obtained immediately after birth are appropriate clinical samples for lung maturity testing.
- The combination of ADSA and BAM provide fast and reliable information on lung maturity by analyses of gastric aspirates from newborns.

Keywords: Lung maturity, Infants, IMinimum surface tension, Axisymmetric drop shape analysis, Brewster angle microscopy.

Acknowledgements

This research was financially supported by the Bulgarian National Science Fund, Ministry of Education and Science (Grant No ДН 03/16).

Proliferation and differentiation of mesenchymal stem cells at implant-tissue interface

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Abstract: The pluripotency of mesenchymal stem cells (MSC) makes them a promising cell source for tissue engineering and regenerative medicine. Their extensive proliferation capacity, multilineage differentiation potential, and ability to evade the immune system have stimulated great interest in the possibility of utilizing MSC in clinical applications [1]. Intriguingly, melatonin (MT), which is primarily secreted by the pineal body, has been found to influence the fate of MSC during various physiological and pathological processes. In general, MT serves as a component of the homeostatic and cell-protective agents, which protect MSC from oxidation, inflammation, apoptosis, ischemia, and aging for regulating MSC differentiation and protection in different organs and tissues [2, 3]. Our aim is to investigate the effect of melatonin on proliferation and differentiation of MSC at implant-tissue interface (TI6AL4V and hydroxyapatite based *in vitro* systems). In our experiments, melatonin in a wide range of concentration had no effect on the proliferation of MSC proved by MTT test. MT effect on osteogenic differentiation of MSC cultured on titanium implants was investigated. Our results proved that MT is a good candidate to improve the behavior of MSC at implant-tissue interface.

Keywords: Mesenchymal stem cells, Implant-tissue interface, Proliferation, Differentiation.

Acknowledgements
The study was funded by Grant DN13/8, National Science Fund.

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Molecular diagnosis of measles virus – advantages and disadvantages

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Abstract:
Introduction: Measles is an acute illness and one of the most contagious human diseases caused by Measles virus (genus Morbillivirus, family Paramyxoviridae). The disease is transmitted via airborne respiratory droplets, or by direct contact with nasal and throat secretions of infected individuals [1]. This study aimed to evaluate the advantages and disadvantages of measles virus RNA detection by PT-PCR to the diagnosis of measles.
Material and Methods: For period of seven years (2013 – 2019) the total 334 patients were tested. Two types of clinical materials were used – sera samples (n=334) and nasal swabs (334). The serological - indirect EIA test for detection of the specific IgM antibodies and molecular methods - extraction and detection of viral RNA were used [2, 3].
Results: The marker (ELISA IgM and viral RNA) for acute measles infection in 277 patients (238 serum samples and 277 nasal swabs) was detected. The rate of detection of the measles virus by molecular method is 83%, compared to 71%, by the serological method. Measles virus can be demonstrated in nasal swabs with greater frequency at the onset of clinical symptoms, and the collection method is less invasive and recommended in young children, infants and pregnant women.
Conclusion: The combined laboratory approach to detect measles in WHO accredited laboratory (immunoenzymatic and molecular assay of each suspected case) is a requisite for measles detection.

Keywords: Measles, RT-PCR, ELISA IgM.

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In vitro study of cytotoxic and anti-proliferative effects of synthetic chalcones against various tumor and non-tumor cell lines

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Abstract: Chalcones (1,3-diaryl-2-propen-1-ones) are open chain flavonoids. During the last decades, numerous investigations were carried out on the pharmacological activities of plant-derived and synthetic chalcones. They possess many biomedical properties. The purpose of the present study was to evaluate *in vitro* cytotoxic and anti-proliferative effects of two groups of synthetic chalcones: two unsubstituted in ring A and two, which possessed 3′,4′,5′-trimethoxy groups. Cytotoxic activity of compounds (0.00001 - 100 mg/mL) was measured against a panel of cell lines: two normal (L20B, mouse fibroblasts; Vero, Green monkey kidney) and two human cancer (RD, rhabdomyosarcoma; Hela, cervix carcinoma) using MTT assay, Trypan blue dye exclusion test and morphometric analysis. Our results demonstrated that three of the tested chalcones (48 and 72h) exert significant anti-proliferative effect on tumor cell lines, mainly in HeLa cells. This effect for one of the unsubstituted in ring A chalcone was observed only at high (100 and 50 mg/mL) concentrations. No cytotoxic activity of two unsubstituted chalcones were observed in non-tumor cells. These data indicate that three of tested synthetic chalcones have cytotoxic and anti-proliferative activities on tested cancer cell lines and may have promise as a source of anticancer agents.

Keywords: Chalcones, Cytotoxicity, Anti-proliferative activity, MTT assay, Cancer.

Redox-sensitive quantum biosensors for cellular and tissues redox mapping

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Abstract: The current study is related to development of new type redox-sensitive probes for detection of redox status in viable cells and tissues for definition of oxidative stress/total redox capacity areas by using advanced imaging techniques. The quantum probes will be applicable for prediction of the "hot spots" (tissues, organs,systems) with impaired redox homeostasis.

Our nanoprobes are chemically based on conjugation of quantum dots(QDs; cadmium-free) with multi-nitroxide-functionalized cyclodextrin (QD@CD-TEMPO; QD@CD-TEMPOH) and combine several features: (i) three imaging modalities (MRI, EPR/ESR, optical); (ii) intracellular delivery and localization into the mitochondria (predominantly) – due to the presence of trimethylphosphonium group; (iii) possibility to sense and visualize the areas of oxidative stress and redox-status in living cells and tissues; (iv) redox-modulating activity.

We demonstrated that quantum sensors enter into viable cells, visualized by EPR and fluorescent imaging. They were successfully applied for detection of redox-processes in model (cell-free) systems and cell lines with different proliferating activity, as well as in animal model of renal dysfunction, based on redox imbalance and oxidative damage, induced by dietary cholesterol.

The proposed redox-sensitive biosensors demonstrate possibilities for redox mapping *in vitro* and *in vivo* and high potential for diagnostics and therapy of variety of "free radical diseases" (e.g., cancer, neurodegeneration, diabetes, atherosclerosis, inflammation, etc.).

Keywords: Free radical diseases, Redox imaging, Oxidative stress, Quantum dots, Nitroxides.

Acknowledgements
This study was partially supported by the JSPS Fellowship, JSPS KAKENHI - JP18F18766 (granted to S.S.) and DFNP 11/2.2017 (granted to B.N. and S.S.).

Catnip (*Nepeta nuda*) photosynthetic response to micropropagation and cryopreservation

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Abstract: Biotechnological approaches such as micropropagation and cryopreservation are useful tools for long-term conservation of medicinal plants with pharmaceutically valuable metabolites [1-3]. The catnip (*Nepeta nuda*) is a plant widely used for its broad spectrum of therapeutic activities and here we apply the chlorophyll fluorescence approach to assess the plant photosynthetic machinery state upon micropropagation and cryopreservation. The efficiency of electron transport chain was quantified by JIP test and showed that during *N. nuda in vitro* cultivation the energy flow absorbed by photosystem II per active reaction center (ABS/RC) increased, while the relative number of reaction centers per unit area (RC/CS0) decreased, which could be due to the lower light intensity in *in vitro* conditions compared to the sunlight in natural environment. In the *in vitro* variant, the photosystems (PIABS and PItotal)' performance decreased. Similar results were obtained for the cryo variant, i.e. *in vitro* plant after freezing. During ex vitro and ex cryo adaptation most of the studied parameters were restored to the *in vivo* level and even a slight increase was observed in the photosystem II performance. We conclude that the *N. nuda* micropropagation and cryopreservation seem to be successful.

Keywords: Chlorophyll fluorescence, *In vitro* cultivation, Medicinal plant.

Acknowledgements
We thank Milena Dimitrova for the technical assistance.

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Laboratory diagnostic of Q-fever positive patients during the first half of 2019

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Abstract: Q-fever, caused by *Coxiella burnetii* is worldwide spread [1]. The aim of the present study was to make a comparative study of sensitivity and specificity of various laboratory methods and clinical samples for early diagnostics of Q-fever among patients with fever of unknown origin, atypical pneumonia, pericarditis and others [2] at the NRL "Rickettsia and Cell Cultures" in the first half of 2019. Serological (ELISA methods) and molecular method (one-step PCR) were used. From all 196 clinical samples, 29 (14.8%) were positive for anti-*C. burnetii* IgM/IgG phase II antibodies, from which 7 plasma (24%) and 22 (75.9%) serum samples. Anti-*C. burnetii* IgG phase I/II antibodies were established in 7 (24%) and 3 (10.3%) samples, respectively. A positive PCR signal (*sodB* region) was detected in 21 (10.7%) serum/plasma samples. From all tested patients, 12 (6%) were children aged ≤ 18 years.

It can be summarized that both of tested methods could be used for the laboratory diagnostic of Q-fever. But only PCR method and plasma were found to be appropriate for the early stage of the infection [3]. This is explained by the specificity in the pathogenesis and the reaction of immunity system against *C. burnetii*, which is an intracellular agent.

Keywords: Q fever, *Coxiella burnetii*, PCR, ELISA, Serum samples, Blood plasma.

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Comparative analysis of the anti-tumor effect of dimethylsphingosine and miltefosine on A549 and HUVEC cells

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Abstract: Miltefosine (HePC) is an alkylphosphocholine which belongs to a group of agents with high selectivity to tumor cells affecting cell membranes and unlocking various signaling pathways, including the pro-apoptotic [1]. N,N-dimethylsphingosine (DMS) is another pro-apoptotic agent which is a specific inhibitor of sphingosine kinase 1 [2-3]. The aim of the present study was to show the effect of the combined treatment with both pro-apoptotic agents on the proliferative potential as well as on induction of apoptosis of human adenocarcinoma cells (A549) and human umbilical vein endothelial cells (HUVEC). The results revealed a synergistic cytotoxic effect of the above agents in the treatment of the cancer cell line while in the treatment of HUVEC such effect was generally lacking. The FACS analysis proved that combined treatment of A549 caused a significantly higher extent of apoptosis when compared to the individual treatments with the anti-cancer agents. For instance, the combined treatment with HePC IC_{50}+DMS IC_{25} showed the highest degree of late apoptotic/necrotic A549 cells compared to the single HePC IC_{50} and DMS IC_{50} treatments (66.11% to 40% and 8%, respectively). The same combination of drugs induced approximately six-fold lower rate of apoptosis in HUVEC (13.4%) which confirmed the cell specific cytotoxicity of the above agents obtained by MTT test. In conclusion, our findings proved the cell specificity of the above combined anti-tumor treatment and revealed for the first time their synergistic cytotoxic and pro-apoptotic effect on lung cancer A549 cells. These findings would be useful in cancer therapy to prevent side effects of the used drugs and in the same time to achieve a sufficient positive effect of therapy.

Keywords: Dimethylsphingosine, Miltefosine, Lung cancer cells, Primary human umbilical vein endothelial cells, Apoptosis, Synergy.

Acknowledgements
This work was supported by Grants DN 11/1/2017 and KP-06-H21/12/2018 from the Bulgarian Science Fund and partially by Contract DO1-154/28/08/2018/ „Scientific Infrastructure on Cell Technologies in Biomedicine (SICTB)".

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Effect of erufosine and simvastatin on cell proliferation and motility of breast cancer cells MDA-MB 231 and MCF-7

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Abstract: Erufosine (erucylphospho-N,N,N,-trimethylpropylammonium, EPC3) is a membrane-operating agent which inhibits the development of the tumor cells inducing changes in the membrane fluidity and modulating the signal transduction pathways originating from the cell membrane [1]. Statins represent a class of lipid-lowering medications that block the conversion of HMG-CoA to mevalonic acid. Simvastatin is a potent competitive inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase. Statin induces apoptosis via JNK-signaling pathway which is affected also by erufosine [2]. Simvastatin shows inhibiting effect against aggressive breast cancer subtypes *in vitro*. We investigated the anticancer activity of erufosine and simvastatin on the MDA-MB-231 and MCF-7 breast cancer cell lines in terms of inhibition of cell proliferation and motility. The effect on the cell proliferation was measured using MTT assay and the alterations on cell motility was investigated by wound-healing assay. Our results strongly suggest that simvastatin and erufosine affect the tumor cell lines MDA-MB 231 and MCF-7 causing inhibition of the cell proliferation and motility in a dose - dependent manner. In addition, the both agents have synergistic effect on the above processes. Based on our results, we can conclude that both agents have appropriate characteristics for their use in anti-tumor therapy [3].

Keywords: Erufosine, Simvastatin, Breast cancer cells, Proliferation, Motility.

Acknowledgements
This work was supported by projects DN 11/1/2017 and KP -06-N21/12/2018 of NSF.

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Session „Excitable Structures and Motor Activity"

Recruitment curves during different levels of co-activation of antagonist muscles in non-dominant hand: A transcranial magnetic stimulation study

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Abstract: The aim of the present study was to compare the effect of different levels of co-activation of antagonist muscles on the recruitment curves during transcranial magnetic stimulation (TMS) in non-dominant left hand. And the secondary purpose was to compare the new collected data with our previous data about recruitment curves in dominant right hand.

Effect of different levels of co-activation of antagonist muscles on motor cortex excitability has been studied in seven right-handed healthy volunteers. Motor evoked potentials (MEPs) were recorded from first dorsal interosseous muscle of non-dominant left hand in response to contralateral TMS during relax, isometric index finger abduction and co-activation of antagonist muscles. The excitability of motor cortex was assessed by the amplitudes of MEPs recorded in responses to increasing stimulation intensity – 100, 110, 120, 130 and 140% of individual measured motor threshold at relax (RMT).

We found significant changes between relax condition and each of the two active motor tasks almost at all investigated TMS intensities. The only exception was between relax condition and co-activation condition at 120% RMT. Also, we found that the recruitment curve at index finger abduction was significantly higher than the recruitment curve at co-activation of antagonist muscles.

Keywords: Transcranial magnetic stimulation, Motor threshold at relax, Motor evoked potential.

Computer vision for assessment of scoliosis from dual energy X-ray (DXA) image

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Abstract:

Introduction: Computer vision is an interdisciplinary scientific field , which include methods for acquiring, processing, analyzing and understanding digital images, and extraction of high-dimensional data to produce numerical or symbolic information. Computer vision is closely linked with artificial intelligence and its goal is not only to see, but also to provide useful results based on the observation. Computer vision technique has shown a great application in assessment of medical images. The aim of this study is to investigate the incidence of lumbar scoliosis from DXA images through computer vision. Scoliosis is defined as curvature in the frontal plain of the body and plays an important role in the quality and the accuracy of the scan results.
Materials and methods: We have assessed 376 DXA lumbar spine scans available in the DICOM format as a raw image. With the help of the openCV library in python we have made an application to process the spine images and determine the angle of the lumbar scoliosis. We have used the Hough Line Transform method to find the lines parallel to the vertebrae and then we calculated the angle between them to find the scoliosis angle /Cobb's angle/.
Results: The mean scoliosis angle of all 376 subjects was 2.97 degrees ± 2.96 degrees. 9 subjects had scoliosis angle greater than 10 degrees – mean angle 14.13 degrees ± 6.21 degrees. The minimum value was 10.02 degrees and the maximum 30.02 degrees. The remaining 367 people had scoliosis angle equal or less than 10 degrees. The mean scoliosis angle of this group was 2.69 degrees ± 2.24 degrees (minimum: 0 degrees, maximum: 9.98 degrees).
Conclusion: This method as a part of computer vision may be useful for the detection of lumbar spine scoliosis and could optimize the DXA scan quality.

Keywords: Computer vision, Scoliosis, DXA.

Session
„Biomedical Engineering"

Method of remote continuous pulse oximetry for screening heart defects

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Abstract: Cardiovascular diseases are the most common cause of death worldwide over the last few decades in the world. Early detection of heart defects can reduce the mortality rate. However, accurate detection of heart diseases [1] in all cases and consultation of a patient for 24 hours by a doctor is not available since it requires more sapience, time, and expertise. In this study, a new method and system for continuous remote pulse oximetry [2] screening are presented. For the accurate monitoring of heart disease, a machine learning technique is proposed which had been derived from a distinctive analysis among several machine learning algorithms. The proposed method allows monitoring the heart disease patient round-the-clock by his/her caretaker/doctor. A real-time patient monitoring system architecture was developed and presented.

Additionally, the pulse oximetry screening of newborns [3] is a way to detect heart problems that might otherwise go unnoticed, and that if left untreated, could be fatal for them. Another important feature of the proposed method was that as soon as any real-time parameter of the patient exceeds the threshold, the patient and the prescribed doctor is notified.

Keywords: Cardiovascular, Pulse rate, Oximetry, Heart defect, Screening.

Acknowledgements
This paper is supported by the National Scientific Program "Information and Communication Technologies for a Single Digital Market in Science, Education and Security(ICTinSES)", financed by the Ministry of Education and Science.

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Development of an active elbow orthosis prototype

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Abstract: Advances in the material sciences, 3D printing technology, bio potential sensors and microprocessor technologies, have led to appearance of orthotic devices with promising functional capabilities, in order to assist physically disabled or elderly people, to increase the limbs 'strength and for self-rehabilitation purposes. The orthosis will allow functional capacity and independence that is not provided without the orthosis. Nowadays there is a big variety of orthotic devices with different structures and control architectures. In contrast to the passive orthoses, the active orthoses have the capacity to increase and in the same time sustain muscle force of the limb, helping the rehabilitation process and users every day activities. This paper describes the development of an active elbow orthosis prototype with the sEMG "on–off" control, appropriate for maximum of two degrees of freedom.

Keywords: Active elbow orthosis, Orthotics, Generalized nets, sEMG data.

Acknowledgements
Work presented here was supported by the Project № DFNP-17-140/01.08.2017 of the Program for Career Development of Young Scientists, BAS.

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Session „Biological Membranes and Biomacromolecules"

Low toxic pharmacologically active compounds/extracts with antineoplastic activity

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Abstract: Malignant tumors are the second more common caused for death in humans worldwide. Despite of the development of new targeted drugs classical cytoreductive chemotherapy remains a standard treatment in many cancer cases. Cytotoxic drugs typically have serious side effects thus limiting their use to eliminate tumor cells. Therefore the discovery of new natural compounds/extracts could help to ameliorate the antineoplastic therapy. Psychrophilic microorganisms successfully colonized deep seas, high mountains and Polar areas. Antarctica offers permanent low temperatures, strong winds, short summer and intensive sun irradiation. To survive under Antarctic conditions microorganisms need to possess adaptable metabolism thus producing bioactive components with attractive pharmacological properties. A methanol extract from bioreactor grown yeast was prepared. Cannabidiol is a natural component in hemp. It has remarkable pharmacological activities such as tumor growth inhibition, pain perception modulation, and anticonvulsive, antipsychotic and antiemetic properties as well. It lacks any psychotropic activity and common toxicity and therefore could be present in food supplements. Our study aimed to determine and compare the antineoplastic activity of the Antarctic yeast extract (*Sporobolomyces salmonicolor* and *Cryptococcus laurentii*) and cannabidiol as pure compound and in hemp extracts against malignant lymphoid cells. The cytotoxic efficacy was measured using the MTT-assay. Induction of apoptosis was ascertained by nuclear changes, DNA fragmentation, PARP cleavage and fragmentation, as well as by caspase activation. Up- and down-regulation of pro- and anti-apoptotic proteins was demonstrated, too. Inhibition of NF-κB was estimated by specific ELISA. Concentration response curves showed IC50 values between 55 and 326 μg/ml for the Antarctic yeast extract and bellow 30 μM for cannabidiol. Nuclear fragmentation and cell cycle changes were demonstrated. Cannabidiol was found to reduce the activity of the NF-κB transcription factor to a comparable with that of curcumin extent.
Cannabidiol and the yeast extract have antineoplastic activity, which is comparable with that of curcumin. Since both natural products are usually well tolerated and do not produce any toxic effects, there is considerable merit in the development of Antarctic yeast and hemp plant extracts as potential therapy for lymphoid neoplasms.

Keywords: Antineoplastic activity *in vitro*, Urothelial cancer, CTCL, Cannabidiol, Antarctic yeast extracts.

Acknowledgements
A substantial part of the experimental work has been supported by the national program BioActiveMed D01-217/30.11.2018, sponsored by the Bulgarian Ministry of Education and Science.

Insights into potential novel biochemical interactions of flavonolignans from *Silybum marianum*: *In silico* and *in vitro* studies

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Abstract: Silybins, the main active compounds of *Silybum marianum* (milk thistle), are well-known for their hepatoprotective properties [1]. More recent *in vitro* and *in vivo* studies demonstrated that silybins and their 2,3 dehydro-derivatives exert anticancer activities, however the mechanisms of these actions are not well defined. In this study the chemical similarity between silybin and 2,3-dehydrosilybin diastereoisomers and drugs in the DrugBank database [2] was evaluated with ROCS software [3]. TanimotoCombo index (TCI) was used to score similarity and it was hypothesized that flavonolignans may interact with the same protein targets as chemically similar drugs do. The similarity with the anticancer drugs vismodegib and vemurafenib was scored with TCI $\geq$ 0.8 and the potential of silybins to interact with their targets, Smoothened homolog and BRAF kinase, respectively, was confirmed by docking. Further *in vitro* studies of the effects of silybins on BRAF V600E kinase activity, and BRAF V600E A375 human melanoma cell line were performed. The preliminary results outline dose-dependent profiles and suggest possible effects of the studied compounds on this target with dehydrosilybins showing stronger effects than silybins. Further experiments are ongoing to more precisely quantify the observed effects. Our results reveal novel anticancer targets for flavonolignans from S*ilybum marianum*.

Keywords: Silybins, Smoothened homolog, BRAF kinase, Cytotoxicity.

Acknowledgements
This work is supported by the Bulgarian Ministry of Education and Science under the National Research Programme "Young scientists and postdoctoral students" approved by DCM # 577/17.08.2018 and by the National Research Programme "Healthy Foods for a Strong Bio-Economy and Quality of Life" approved by DCM # 577/ 17.08.2018.

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Development of a protocol for virtual screening of PPARγ weak partial agonists: Case study on naturally-derived triterpenoids

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Abstract: Triterpenoids are well known metabolic syndrome (MS) modulators. One of the suggested molecular mechanisms of action involves peroxisome proliferator-activated receptor gamma (PPARγ) binding [1-3]. In this study we aimed to: (i) develop a virtual screening (VS) protocol for PPARγ weak partial agonists, (ii) predict potential metabolic transformations of naturally-derived triterpenoids, and (iii) perform VS of the triterpenoids and their metabolites. The NIH PubMed system was searched for naturally-derived triterpenoid saponins and sapogenins which are agonists or up-regulators of the nuclear receptor PPARγ. Structure- and ligand-based approaches were combined in the development of the VS protocol. Metabolites were predicted using Meteor Nexus system (Lhasa Limited). An in-house virtual library of naturally-derived triterpenoids was generated from literature data. A pharmacophore-based docking protocol was developed and applied for VS of the collected triterpenoids. Most of the docking poses reproduced the binding mode of caulophyllogenin (a weak partial agonist) in the complex with PPARγ (PDB ID 5F9B). Our results suggest possible weak partial agonistic activity of the investigated triterpenoids. This research contributes to the mechanistic explanation of the effects of triterpenoids by a potential PPARγ-mediated mode of action and can direct further studies of these compounds for their use as MS modulators.

Keywords: PPARγ, Weak partial agonism, Virtual screening, Triterpenoids, Metabolites, Metabolic syndrome.

Acknowledgements
The prediction of Phase I metabolism was supported by the Bulgarian Ministry of Education and Science under the National Research Programme "Healthy Foods for a Strong Bio-Economy and Quality of Life" approved by DCM # 577/17.08.2018; The development of in-house virtual library of triterpenoids and the virtual screening were supported by the National Science Fund of Bulgaria (grant DM01/1/2016).

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Atomic force microscopy imaging of ultrastructural changes during ageing of red blood cells derived from women with miscarriages and healthy pregnant women

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Abstract: The ageing of red blood cells (RBCs) is of considerable interest in both health and disease states. The morphology of RBCs as well as their membrane surface roughness are associated with the functional status of the cells and are important marks of health condition. In this study, we applied atomic force microscopy imaging to examine the ultrastructural changes during ageing of RBCs derived from women with miscarriages and healthy pregnant and non-pregnant women.

Freshly isolated RBCs from healthy pregnant and non-pregnant women does not differ from those of women with miscarriages and has a typical biconcave structure. Significant changes in the cells shape are observed with ageing, from almost completely biconcave through different morphological classes and complete loss of the normal shape, accompanied by a gradual decrease in the size and membrane roughness of the cells. The ultrastructural changes of RBCs from women with miscarriages occur much earlier during ageing compared to the control ones and the roughness is reduced exponentially during the first 10 days and has lower values than the controls for which the roughness decreases linearly over time.

Our data demonstrate accelerated ageing of erythrocytes derived from women with miscarriages as compared to those from healthy ones.

Keywords: Red blood cells, Atomic force microscopy, Cells ageing, Miscarriages, Membrane roughness.

Acknowledgements

This work is supported by grant KP-06-H21/4, Competition for financial support for basic research projects – 2018, National Science Fund.

Equipment of research infrastructure INFRAMAT under contract D01-155/28.08.2018 (part of Bulgarian National roadmap for research infrastructures) supported by Bulgarian Ministry of Education and Science was used in this investigation.

Calorimetric study of the ageing of erythrocytes derived from women with miscarriages

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Abstract: Thermodynamic approach was applied to study the thermal stability of the major protein constituents (the oxygen-transport metalloprotein hemoglobin (Hb) and the cytoskeletal proteins – spectrin and band 3) of erythrocytes (RBCs) derived from women with miscarriages, non-pregnant and healthy pregnant women during ageing.

The ageing of RBCs for healthy pregnant and non-pregnant (control) groups is characterized with: (i) slight destabilization of Hb, i.e. a shift of Hb thermal tranisiton to lower temperatures; (ii) decrease of the excess heat capacity of Hb thermal transition; (iii) disappearance of band 3 protein transition after the 30th day of storage. These alterations in RBCs thermal stability in the course of ageing happened more rapidly and the band 3 protein transition disappeared earlier for women with miscarrieages than the control groups.

RBCs preheating to 60 °C, that exerts similar effect on Hb stability as erythrocytes' ageing, results in stronger destabilization of Hb for women that underwent miscarriage than for control ones.

Data suggest a correlation between the erythrocytes' thermodynamic properties and the clinical status of women with miscarriages.

Keywords: Erythrocytes, Miscarriages, Differential scanning calorimetry, Cells ageing, Hemoglobin, Structural proteins.

Acknowledgements
This work is supported by grant KP-06-H21/4, Competition for financial support for basic research projects – 2018, National Science Fund

Ketoprofen-based ionic liquids – interactions with bovine serum albumin

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Abstract: Ketoprofen (KP) is a non-steroidal anti-inflammatory drug, an inhibitor of cyclooxygenases [2]. Some of its side effects such as water retention, gut irritation, low bioavailability, and others are due to its low water solubility [2]. Novel formulations based on ionic liquids (ILs) containing an active-pharmaceutical ingredient as a cation or anion can be an alternative to classical drugs and may overcome some of the problems related to solubility and polymorphism [1]. Here we report the synthesis and the characterization of a series of ILs containing a cation amino-acid alkyl ester (AAE) and an anion KP. The binding sites and the binding constants of the KP and KP-based ILs with bovine serum albumin (BSA) were estimated by fluorescence spectroscopy following the quenching of the tryptophan fluorescence. The estimated binding constants for the KP-ILs were between $1{,}5.10^5$ and $8{,}2.10^5$ $mol^{-1}L^{-1}$ and were in the same range as the binding constant estimated for KP ($2{,}6.10^5$ $mol^{-1}L^{-1}$). The effect of the KP-ILs on the secondary structure of BSA was assessed by monitoring the changes in the Amide I band in the ATR-FTIR spectra of the BSA-IL complexes in comparison to the spectrum of BSA-ketoprofen. In general, in the presence of the ILs, we observed a decrease in α-helical content on behalf of β-structures.

Calorimetric studies have shown that in the tested concentrations KP-ILs produces the same effect on albumin thermal stability as those observed for KP.

Keywords: Ketoprofen, Ionic liquids, Bovine serum albumin, Secondary structure, Fluorescence spectroscopy, Differential scanning calorimetry.

Acknowledgements
This work was partially supported by the Bulgarian Ministry of Education and Science under the National Research Programme "Young scientists and postdoctoral students" approved by DCM # 577/17.08.2018.

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Membrane molecular packing and domain size changes induced by oxidized lipids

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Abstract: Lipid oxidation plays a role in inflammatory processes related with various diseases, such as Alzheimer disease, atherosclerosis, cardiovascular diseases, cancers among others. As prone to oxidation, polyunsaturated lipids are of obvious importance when attempting to understand the consequences of oxidative stress on membrane-associated interactions and processes. To examine the physicochemical changes in membrane lateral organization induced by oxidized lipids we prepared model membranes that mimic the lipid composition of specialized cellular membrane domains, called rafts. These domains are essential for a number of cellular functions and enriched in sphingolipids and cholesterol. Rafts in model membranes appear as a liquid-ordered (L_o) phase surrounded by continuous liquid-disordered (L_d) phase. The aim of the present study is to investigate the effect of one of the most physiologically active oxidized lipids 1-palmitoyl-2-(5'-oxo-valeroyl)-*sn*-glycero-3-phosphocholine (POVPC) on the membrane molecular packing and the size of nanorafts. These characteristic parameters of the membrane organization were studied as a function of the degree of fatty acid unsaturation at *sn-2* position in phosphatidylcholine molecule. Monounsaturated lipid, palmitoyl-oleoyl phosphatidylcholine (POPC), versus polyunsaturated one, palmitoyl-docosahexaenoyl phosphatidylcholine (PDPC) was compared in different types of mixtures with and without sphingomyelin and cholesterol. Laurdan spectra were measured to assess membrane molecular packing induced by POVPC whereas the raft sizes were estimated by using spectroscopic DPH-TEMPO method. POVPC induced larger changes in membrane organization in monounsaturated lipid matrix compared to polyunsaturated one. The oxidized lipid modified rafts sizes with potential effects on cellular signaling.

Keywords: Lipid rafts, Domain size, Oxidized lipids, DPH, Laurdan.

Acknowledgements
This work was supported by Project № DM 11/1 14/12/2017.

Antioxidative and packing properties of plant polyphenolic compounds on artificial and cell plasma membranes studied by fluorescence methods

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Abstract: Proper structure and function of cell membrane are crucial for cell vitality. Small and dynamic clusters of lipids in a more ordered state (rafts) mediate essential cellular processes. Lipid oxidation in plasma membrane is one of the oxidative stress pathways, which perturb normal cell functions.

The present day's research in pharmacology is focused on substances with plant origin. Some biological activities of plants from *Inula* species are attributed to the presence of flavonoid glycosides (FG) and phenolic acids (PA). In this study, we tested these polyphenolic compounds, extracted from *Inula oculus-christi,* on membrane fluidity changes. Detection of the lipid oxidation process was accomplished by monitoring the decrease in fluorescence intensity of *cis*-parinaric acid in artificial membranes. The effect on membrane packing was evaluated by using two fluorescent probes reporting lipid order and fluidity (Laurdan and DPH). The impact of FG and PA to modulate raft formation was assessed by fluorescence quenching method (DPH-Tempo). Polarity sensitive fluophore di-4-ANEPPDHQ was applied for probing the degree of membrane packing in two cell lines (MDCK II and A549) induced by FG and PA.

Both extracts exerted antioxidative activity over model membranes. Fluorescence quenching method revealed increasing of fraction of raft-like domains in liposomes by the extracts. GP values of di-4-ANEPPDHQ acquired by micrographs showed that the overall structure of treated MDCK II cells exhibited more fluid membranes than controls. Different trend was observed in A549 cells when treated with FG and PA. FGs made the membranes more ordered whereas PA fluidized them. We suggested a mechanism based on structural and membrane organization changes induced by studied polyphenolic compounds that imply beneficial effect on human health.

Keywords: Flavonoid glycosides, Phenolic acids, Rafts, Oxidative stress, Antioxidants.

Acknowledgements
This work was supported by projects DM 11/1/2017, DN 09/11/2016 and DN 18/15/2017.

Effect of single walled carbon nanotubes on the photosynthetic apparatus of pea plants

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Abstract: Nanomaterials are gaining an ever wider application in agriculture (nanoagronomy) with the aim to increase plant yields, resistance to different biotic and abiotic factors, and to decrease soil contamination. Their effect on photosynthesis however is not thoroughly investigated yet.

In this work we study the mode of interaction of single walled carbon nanotubes (SWCNT) stabilized by Pluronic P85 copolymer with the photosynthetic apparatus of pea plants after foliar application. Low doses (10 mg L^{-1}) of SWCNT did not exert inhibitory effect on the photosynthetic function, but induced the formation of higher grana. Higher concentrations (100 and 300 mg L^{-1}) significantly reduced the net photosynthetic rate, water use efficiency and quantum yield of photosystem II and led to chloroplast structural modifications related to grana stacking and macroorganization. The latter changes were associated with decreased deepoxidation state and altered kinetics of non-photochemical quenching, demonstrating that the SWCNT treatment affected the plant photoprotection capability.

Our data strongly suggest that SWCNTs (added in low concentrations) could be promising nanocarriers for bioactive molecules that might help produce climate-resilient crops with improved yield and nutritional value.

Keywords: Single walled carbon nanotubes, Photosynthesis, Chloroplast structure, Nanoagronomy, Nanocarriers.

Clarifying the causes for different salt tolerance of *Sorghum* ssp. and *Zea mays*

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Abstract: Salinization is one of the most important global environmental problems, which limits plant growth and development and thus decreases the crop yields**.** The aim of this study was to investigate the impact of salinity on the pigment composition and the functions of the photosynthetic apparatus of *Zea mays (ES Farady* and *Mayflower*) and *Sorghum* (*BMR Gold* and *ES Foehn*), grown in Hoagland solutions with different concentration of NaCl (0 – 300 mM) for seven days. The effects of NaCl on *Zea mays* and *Sorghum* plants were assessed by measuring of PAM chlorophyll fluorescence, P_{700} photo-oxidation and pigment content. Data revealed that treatment with high NaCl concentration led to changes in pigment composition, an influence of the efficiency of the photochemical energy conversion (Φ_{PSII}), decrease the photochemical quenching (q_P), the linear electron transport rate (ETR), the maximum (Fv/Fm) and the effective (Fv'/Fm') quantum yields of PSII as well as the restriction of the electron flow from Q_A to plastoquinone. The analysis of P_{700} photo-oxidation suggested that the NaCl induced changes in the both populations of PSI. The different sensitivity to high salt concentrations of the studied plants and the possible adaptation mechanisms to salt stress will be discussed.

Keywords: Maize, Salt stress, *Sorghum*, Chlorophyll fluorescence, Pigments.

Acknowledgements
This work was supported by the Bulgarian Ministry of Education and Science under the National Research Programme "Young scientists and postdoctoral students" approved by DCM # 577/17.08.2018.

Influence of zinc oxide nanoparticles on the photosynthetic apparatus of *Pisum sativum* under salt stress

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Abstract: The aim of this study was to investigate the impacts of zinc oxide nanoparticles (ZnO NPs) on the photosynthetic apparatus of *Pisum sativum* grown under salt stress. Zinc oxide nanoparticles (200 mg/L and 400 mg/L) were applied through foliar spray on the pea plants and NaCl treatment (100 mM) was applied to roots through the nutrient solution. The simultaneous treatment of the pea seedling with ZnO NPs and NaCl was carried out for 14 days. Pulse amplitude modulated chlorophyll fluorescence (PSII functions), P700 photo-oxidation measurements (PSI photochemistry), pigments content and membrane electrolyte leakage (indicator for membrane integrity) were used to assess the effect of ZnO NPs under salt stress on the photosynthetic apparatus. The results showed that ZnO NPs decreased the effect of NaCl treatment on the function of the photosynthetic apparatus, pigment composition and reduced the membrane electrolyte leakage. In addition, the experimental results also revealed that under physiological conditions, ZnO NPs do not influence the studied parameters. Data clearly showed that ZnO NPs mitigate the effects of salt stress on the photosynthetic apparatus of *Pisum sativum*.

Keywords: Zinc oxide nanoparticles, Photosynthetic apparatus, Salinity stress.

Acknowledgements
This work was supported by the Bulgarian Ministry of Education and Science under the National Research Programme "Young scientists and postdoctoral students" approved by DCM # 577/17.08.2018.

Encapsulation of the peptide-based drug opiorphin in alginate beads coated with natural and synthetic hybrid polyelectrolyte-graphene oxide multilayers

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Abstract: Opiorphin (Oph) is a naturally produced endogenous peptide with a strong analgesic effect, superior to that of morphine, and without the severe side effects that morphine and morphine-like drugs exert. However, despite the strong therapeutic potential of opiorphin, its short duration of action, probably due to its low chemical stability and rapid degradation by the peptidases in the bloodstream, represents a serious obstacle to its use into clinical practice.

In this work a variety of natural and synthetic polymers have be explored for Oph capsules' preparation. Most of the already established encapsulation strategies were found unsuitable for Oph loading.

We developed a novel approach to prepare Oph loaded particles. Alginate, a naturally occurring anionic polysaccharide, gel beads were coated with diverse hybrid polyelectrolyte-graphene oxide (PE-GO) multilayers, varying the type of the building polymer couples used (chitosan / hyaluronic acid (Chi / HA) and Poly (allylamine hydrochloride) / Poly (styrene sulfonate) (PSS /PAH)); GO being the last layer; the stability of particles was improved by chemical cross-linking.

We expect that the successful encapsulation of opiorphin in biodegradable particles will result in the development of adequate drug delivery system with effective and prolonged analgesic activity and will provide a new alternative for pain management.

Keywords: Opiorphine, Alginate beads, Polyelectrolyte films, Graphene oxide.

Acknowledgements
This work is supported by project DAAD Austria 01/7, 2017, bilateral collaboration with Austria, National Science Fund.

Втора научна сесия
„Биомедицина и качество на живот“ 2019

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