Session 12: Analytics in biomedicine

Lectures

L12.1

Novel multisensor platforms for biomedical analysis of electrolytes and monitoring of ion-transport through biological membranes

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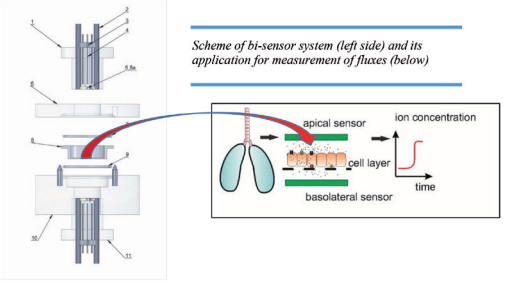
Multielectrode sensor arrays and bi-sensor systems designed for short and/or real-time concentration measurements of sodium, potassium, chloride, bicarbonate and pH are presented. The systems used for routine measurements in a small volume of blood and in biological liquids bathing a living human bronchial epithelial cell monolayer to characterize ion-fluxes are in focus [1, 2].

Several application-driven research milestones allowing realization of ion-senors idea will be characterized. They include inventing solid-contact ion-selective electrodes, junctionless reference electrodes, one-drop measurement, and signal interpretation by Nernst-Planck- Poisson model. Support provided by recommendations for clinical diagnostic measurements will be as well employed.

Very recent results on dual-function reference electrodes that work as the electrochemically active body for ion-sensors and biosensors will be used to show new perspectives and challenges.



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L12.2

Two faces of transcription factor SNAIL

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Rhabdomyosarcoma (RMS) is a frequent non-epithelial tumor of soft tissue that causes death and morbidity of young patients. Despite numerous efforts, the precise mechanism of RMS development is unknown. Our studies demonstrate for the first time that SNAIL, a transcription factor regulating the epithelial to mesenchymal transition in tumors of epithelial origin, is also a crucial regulator of RMS development, growth and differentiation. We found that the expression of the SNAIL transcription factor is elevated in the aggressive alveolar subtype of RMS, characterized by a low myogenic differentiation status. Interestingly, the differentiation of human RMS diminishes the SNAIL level. Moreover, SNAIL silencing completely abolishes the growth of human RMS xenotransplants. We discovered that SNAIL inhibits myogenic differentiation of RMS by binding to the MYF5 promoter, suppressing its expression, and displacing MYOD from its canonical to alternative E-box sequences. SNAIL silencing allows the re-expression of MYF5 and canonical MYOD binding, with both actions promoting RMS cell myogenic differentiation. Our data clearly suggest that SNAIL is a key regulator of muscle differentiation. We described novel, unexpected molecular mechanisms of direct SNAIL-mediated regulation of myogenic factors that expands the diverse set of SNAIL activities. These novel results open potential avenues for the development of innovative therapeutic strategies of RMS based on SNAIL silencing.

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Oral presentations

O12.1

Microbial profiling of 1000-2000 year old human remains

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The advances in the NGS sequencing have revolutionized the field of paleogenomics. Technological and methodological improvements allowed for the reconstruction of genomes of long-dead organisms. Typically, the studies of ancient DNA (aDNA) isolated from human or animal remains are focused on the endogenous aDNA only. However, most reads in those NGS datasets do not originate from the studied individual, but from microorganisms that colonized a sample *postmortem*.
We employed metagenomics analysis to study comprehensively microbial composition of ancient human remains. 161 samples dated to 1-1200AD from seven archaeological sites in Central Europe and of different storage conditions were analyzed. The majority of identified microbes were ubiquitous environmental species that most likely colonized the host remains not long ago. In line with those results we showed that there is no correlation between specific prokaryotic taxa and temporal or geographical origin of the samples. Instead we noticed that the microbial profile is unique for an individual. Bacteria and archaea species characteristic for human oral and gut flora, as well as potential pathogens, were identified in two-thirds of the samples. The genetic material of human associated bacterial species revealed a typical for aDNA damage pattern at a degree comparable with endogenous human aDNA.
Our work maximizes the amount of information that can be obtained from ancient samples. We evaluated the impact of micro-environments on microbial composition and propose a workflow which permits to determine the source and taxa of exogenous contamination (human-related/soil prokaryotes) and to estimate the potential age of microbial aDNA. Based on the collected data we were able to distinguish between human associated, pathogenic or non-pathogenic bacteria, and microbial contamination from the surrounding environment. This further improves authentication process of potential ancient pathogens in particular.

Posters

P12.1

Gel electrophoresis in a polyvinylalcohol coated fused silica capillary for assessment of oligoribonucleotides

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Nowadays, the development of capillary electrophoresis (CE) plays a key role in bringing about the state-of-art application of DNA and RNA analysis. CE is a special method where an electrical field is used to separate the components of a mixture [1] in accordance with their charge to size ratio. The merit of the capillary separation is laid down in a narrow silica tube inside which the separation is carried out and the signals are subsequently on-line detected. Capillary gel electrophoresis (CGE) with a viscous polymer solution as a separating matrix has emerged a powerful tool for the analysis of synthetic oligonucleotides [2]. Application of the poly(vinyl) alcohol-coated (PVA-coated) capillary in CGE gives a possibility of selective separation of oligoribonucleotides and their modifications with high resolution. Herein we present a study on PVA-CGE usage for the separation of short-chain synthetic RNA from their phosphorylated analogs and for analyzing of oligomers of mixed sequences. Moreover, quality assessment and structure determining of shorter oligomers alike small interfering RNA (siRNA) is also depicted and elucidated, since they have become essential for ribonucleic acid technology and have been more often applied in medical applications[3]. The usefulness of the CGE with a PVA-layered capillary is presented as an alternative to conventionally used techniques.

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1. Whatley H (2001) Basic Principles and Modes of Capillary Electrophoresis in Clinical and Forensic Applications of Capillary Electrophoresis, Petersen JR, Mohammad AA, eds. Chapter 2, pp 21-58, Humana Press Inc. Totowa, NJ, USA.
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P12.2

Qualitative and quantitative amino acid analysis in serum, plasma, urine and cerebrospinal fluid by liquid chromatography-electrospray mass spectrometry – LC-ESI-MS/MS

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Introduction: Amino acids as molecular building blocks of proteins also play an important role in many metabolic pathways. Determination of correlations and concentrations of individual amino acids is clinically significant factor in determining the risk of disease. Currently there are known many diseases associated with amino acids metabolic disorders, among others: branched-chain amino aciduria (maple syrup urine disease), branched-chain organic acidurias, phenylketonuria, nonketotic hyperglycinemia, homocystinuria, urea cycle defects, defects of biotin metabolism, disorders of glutathione metabolism, disorders of GABA metabolism [1]. Many of the amino acids and their derivatives are now recognized as markers or potential markers of diseases, however, many of the pathways require further studies. Therefore, fast and reliable methods of amino acids analysis in plasma, serum and cerebrospinal fluid are necessary for the diagnosis and therapy of metabolic disorders associated with amino acids.
Materials and methods: For the analysis of metabolic disorders of amino acids we have used the following matrices: serum, plasma, urine and cerebrospinal fluid. Chromatographic separation of amino acids was achieved using a nanoAcquity UPLC system. The MS/MS measurements were carried out using a Xevo G2 Q-TOF mass spectrometer in the positive ion mode. For the qualitative and quantitative analysis MassLynx and QuanLynx software from Waters were used.
Results and Discussion: The purpose of this studies was to develop a method of the simultaneous extraction and derivatization of amino acids from biological samples. Achieving this method allowed us to obtain a large range of linearity, high stability of extracted substances from the samples and the appropriate level of *limit of detection* (LOD) and *limit of quantification* (LOQ). The use of mass spectrometer Q-TOF coupled with UPLC system provides detailed and comprehensive qualitative information necessary for the proper data analysis.

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P12.3

Correlation between serum 25-hydroxyvitamin D and selected clinical and immunological parameters in patients with relapsing-remitting multiple sclerosis – a pilot study

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Introduction: Scientists have suggested that the vitamin D deficit is associated with many autoimmune diseases, including multiple sclerosis (MS). The worldwide prevalence of multiple sclerosis is correlated with exposure to UVB radiation. However, the potential role of vitamin D in MS course has not been clarified yet and the studies have revealed controversial results.
The aim of the current study was to estimate correlation between serum 25-hydroxyvitamin D_3 level and disease activity evaluated by: magnetic resonance imaging (MRI), the degree of physical disability (EDSS), neopterin concentration (a marker of cellular immune response), and microtubule-associated protein tau concentration (MAPT, a marker of neurons degradation).
Material and methods: The study comprised 20 patients suffering from relapsing-remitting multiple sclerosis – RRMS (aged 44.44±12.67, mean EDSS 5.81±1.19) as well as 10 healthy, age-matched volunteers. The serum concentration of 25-hydroxyvitamin D_3 was determined by means of the HPLC method, whereas concentrations of neopterin and MAPT were measured with the use of the immunoenzymatic method ELISA.
Results: The concentration of 25-hydroxyvitamin D_3 in RRMS patients did not differ significantly in comparison to healthy controls (18.89±11.27 ng/mL *vs.* 21.63±9.41 ng/mL, respectively). However, in 85% of RRMS patients (17/20) a full (20 ng/L) or moderate (20-30 ng/mL) deficit of vitamin D was found. Neopterin concentration was significantly higher in RRMS patients vs. control group (21.78±7.89 nM/L *vs.* 4.94±1.01 nM/L, $p<0.001$). The mean concentration of tau protein in RRMS group was 97.67±78.9 pg/Ml, whereas in all the control group MAPT cocentation was below the detection limit (7.8 pg/mL). In RRMS group significant inverse correlations between 25-hydroxyvitamin D3 level and: neopterin conentration (r=–0.18, $p<0.05$), EDSS score (r=–0281, p0.01) and the number of lesions on MR image (r=–0.63, p0.001) were sown.
Conclusions: The obtained results seem to confirm the important role of vitamin D in relapsing-remitting multiple sclerosis. The study will be continued to evaluate the efficacy of vitamin D supplementation in patients with multiple sclerosis.

P12.4

The effect of glycation on the bovine serum albumin conformation and ligand binding properties with regard to gliclazide and cilazapril

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Background: Increased glucose concentration, which is the most characteristic symptom of diabetes, leads to many biochemical disturbances, causing also structural changes of serum albumin, the main drug career in human organism. **Aim**: In present study we evaluated the effect of glycation on the secondary and tertiary structure of albumin and its binding properties towards gliclazide and cilazapril, which although evidenced interactions are commonly used in the treatment of diabetic hypertensive patients. **Materials and methods**: Bovine serum albumin (BSA) solutions, both nonglycated (native) and glycated: by 10 mM glucose (gly-10BSA) and by 30 mM glucose (gly-30BSA), were prepared, simulating physiological and diabetic conditions. The interactions of gliclazide, sulfonylurea derivative with hypoglycemic action with native and differently glycated albumin, as well as its combination with cilazapril, an angiotesin converting enzyme inhibitor demonstrating hypotensive effect, were studied. The structural changes of BSA were assessed by fluorescence and circular dichroism spectroscopy. **Results**: We observed strong quenching of the fluorescence at 280 nm excitation wavelength, what clearly indicates that the binding of the gliclazide to BSA changed the microenviroment of tryptophan residue and the tertiary structure of BSA, both nonglycated and glycated. The binding constants (K_a) decreased with the increasing degree of glycation (1.98, 1.72, 0.42×10^4 M^{-1} for native, gly-10BSA and gly-30BSA, respectively). In presence of cilazapril, further decrease of K_a was observed (0.448, 0.255 and 0.236×10^4 M^{-1}, as above). It was accompanied by simultaneous slight changes in alpha-helix structure (from 1.72% to 5.63%). **Conclusions**: The affinity of the glycated albumin for gliclazide was slightly reduced in physiological model of glycation and markedly reduced in diabetic model. Interestingly, the effect of cilazapril on BSA-gliclazide binding was the most evident in native model (over fourfold decrease), while in diabetic model was less than twofold. Our study indicates clearly that albumin glycation influences its binding properties, what should be considered in management of diabetes.

P12.5

Electrochemical identification of the novel glutathione conjugates of antitumor-active acridinones, 2-hydroxacridinone and C-1305

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The combination of electrochemistry coupled on-line to mass spectrometry (EC/MS) has been shown to be a quick and convenient analytical technique for the simulation of some types of oxidative metabolism reactions and for studying the formation of reactive metabolites. The present work is a part of a wide research project aimed to elucidate the molecular mechanism of oxidative activation of the antitumor-active imidazo- (IA) and triazoloacridinone (TA) derivatives, C-1311 and C-1305, respectively, the first selected for clinical trials.

The biotransformation pathways of C-1311 and C-1305 were investigated by EC/MS. 2-Hydroxyacridinone (2-OH-AC), their reference compound, was chosen as a test compound to develop and evaluate the electrochemical method. Additionally, the studied compounds have been studied by cyclic voltammetry and controlled potential electrolysis to investigate the ability of acridinone derivatives to undergo oxidative metabolic activation in the living organism. The products of exhausted electrolysis of 2-OH-AC, C-1311 and C-1305 alone and in the presence of glutathione (GSH) were analysed by RP-HPLC with UV-Vis detection and/or diode array and multiple wavelength detection.

The electrochemical oxidation of all acridinones in an electrochemical thin-layer cell equipped with a glassy carbon working electrode was successfully achieved. Moreover, it unveiled the generation of a reactive quinoneimine-form metaboliteof 2-OH-AC (m/z 210) which further reacted with GSH and/or N-acetylcysteine to form adducts (m/z 517 and 373, respectively) *via* the thiol group. The identical GSH conjugate of 2-OH-AC has been identified after controlled potential electrolysis as well as has been detected in conventional *in vitro* metabolism studies with human and rat liver microsomes. We observed that reactions occurring between 2-OH-AC and GSH under catalysis by cytochrome P450 enzymes in liver microsomes was NADPH-dependent. One type of reactive metabolite of C-1305, formed through EC oxidation, was trapped by GSH to form adducts ion with m/z 420, but it was not detected from liver microsome incubations.

In conclusion, EC/MS is a promising tool for the identification of both stable and reactive metabolites in drug development. The obtained results will be useful for further studies on the metabolic transformation of antitumor IA and TA drugs.

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