FROM MEDICINE TO BIONICS

1st European Ph.D. Conference, Budapest, 2013

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Budapest, Hungary 13-15, June 2013

Budapest Novotel Budapest Centrum* * * *

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Budapest March 11th 2013

Dear Colleagues,

It is a great pleasure for us to welcome you to the *From Medicine to Bionics – 1st European PhD Conference.*

As John Selye writes in his famous work (*The Stress of Life*, New York, transl. 1966, p. 63), "The primary purpose of medicine is to reconnoiter the peculiarities of illness, because only based on this information is one able to breast against it. We know that the limited human mind cannot reach the fullness of this knowledge even by the fiercest effort. However, because this mysterious enigma is vitally important for everyone, every small step which moves toward the solution is beneficial." Almost fifty years have passed since the "father" of the "Stress-Theory" composed these sentences. Nevertheless, these words still hold great meaning today, and may serve to inspire all those participating in today's International Congress. This inspiration can in turn motivate those researchers who would like to know more about the human body even down to the molecular level, to achieve new pharmaceutical results in order to take part in the fight against old and newly discovered sicknesses, to make analysis in the field of medicinal chemistry, and to combine the medical sciences with information technology.

We need the collaboration of different disciplines, because the combination of classical medicine, molecular biology, medicinal chemistry, pharmacology, nano-scale opto-electro-magnetics, electronics and computing, and neuroscience is essential in today's work. It is not enough for you to be good in research, you must also excel in your field, because there are many questions which need to be answered! You must learn how to cooperate and work in a team, otherwise the new generation of scientists will not be able to continue that marvelous and heroic work which has made possible for you this new type of natural scientific research, based on multidisciplinary principles and instruments.

American astrophysicist Carl Sagan once said: "Science is far away from that ideal stage that itself to be the perfect instrument of the Knowledge, however the Science is the best which we really have." (The demon-haunted world: science as a candle in the dark, New York 1995, p. 29). The goal of this PhD conference is twofold. On the one hand we seek to summarize some of the corner stones of the current research in the field of medicine, pharmacy and bionics through some significant basic presentations. On the other hand, we wish to give PhD scholars the opportunity to present their projects, analyses, concepts, and results.

It is a distinguished honour for Semmelweis University and Pázmány Péter Catholic University to jointly organise and host this conference. The individual and group projects you will present here today enrich the whole of science and make it possible to help, whether now or in the future, many people around the world. Moreover, it will allow you to face the new challenges of your research field and the international evaluation of your scientific results.

With these words, we wish you successful preparation, a fruitful conference, and a most pleasant stay in Budapest,

Dr. Szabolcs Szuromi
rector of Pázmány Péter Catholic University

Dr. Ágoston Szél
rector of Semmelweis University

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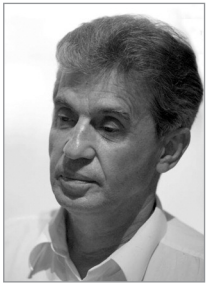

Tamás Roska
Pázmány Péter Catholic University

VENUE

Novotel Budapest Centrum****
Address: Rákóczi út 43-45. 1088 Budapest, Hungary
Web: www.novotel.com

REGISTRATION

Registration desk:
The registration desk is to be found on the ground level of the Novotel Budapest Centrum.

Registration desk opening hours:
Thursday, 13, June, 2013: 17:00 – 20:00,
Friday, 14, June, 2013: 07:30 – 18:00,
Saturday 15, June, 2013: 07:30 – 14:00,

Meeting materials will be available at the registration desk at the conference venue during the conference.

Delegate registration fee includes: access to scientific sessions and exhibition areas, congress bag, abstracts in electronic format, welcome reception on Thursday, concert, banquet dinner on Friday, all coffee breaks plus lunches on Friday and Saturday.

Accompanying person's registration fee includes: access to scientific sessions and exhibition areas, congress bag, welcome reception on Thursday, concert, banquet dinner on Friday, all coffee breaks plus lunches on Friday and Saturday.

SOCIAL PROGRAMS

Banquet Dinner in Domonyvölgy
Friday 14, June 19:00
Departure: By bus transfer from Congress

Venue
Departure time: 19:00
Meeting point: registration desk
Participants and accompanying persons can meet in a relaxed atmosphere and enjoy Hungarian hospitality, cuisine and original folk music. Transfer is included.

CONGRESS OFFICE

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PROGRAM

13 June, Thursday

18:00–19:00 **Registration, Placement of posters**

19:00 **Welcome reception**

14 June, Friday

08:30–09:00 **Opening ceremony**
Prof. Szabolcs Szuromi
Rector, Péter Pázmány Catholic University, Budapest
Prof. Ágoston Szél
Rector, Semmelweis University, Budapest

09:00–09:45 **Plenary lecture I.**
Philip Purnell
Strategic Business Manager, Thomson Reuters
Thomson Reuters New Trends in Research Evaluation & Management

09:45–10:15 **Invited lecture I.**
Prof. Edit Oláh
National Institute of Oncology, Budapest
Research and Genetic Testing in Breast Cancer Predisposition

10:15–10:45 **Coffee break**

10:45–11:30 **Plenary lecture II.**
Prof. Hartmut Neumann
University of Freiburg
Molecular Genetics Meets Epidemiology
Critical Approach for Prevalence Calculations in Rare Disease

11:30–12:00 **Invited lecture II.**
Prof. Zsuzsanna Helyes
University of Pécs
How can Pain and the Effect of New Analgesics be Investigated in Animal Models?

12:00–12:30 **Invited lecture III.**
Prof. Béla Urbányi
Szent István University
For Potential Risks and Side-Effects Please Contact with a Zebrafish

12:30–13:00 **Invited lecture IV.**
Prof. Stefano Alcaro
University Catanzaro
Computational Methods in Drug Discovery: Diffusion in the Scientific Community and Successful Experiences

13:00–14:00 **Lunch**

14:00–14:45 **Plenary lecture III.**
Prof. Anna Fadda
University of Cagliari
"How to Improve the Therapeutic Profile of Drugs by Delivery Systems?"

14:45–15:45 **Poster presentation and discussion**

14:45–16:15 **The Magister Project's final press conference (only for Press)**

15:45–16:15 **Coffee break**

16:15–17:00 **Plenary lecture IV.**
Prof. Gyöngyi Szabó
University of Massachusetts Medical School
Virus and Host Interactions in Hepatitis C virus Infection: the Role of Innate Immunity and micro-RNAs

17:00–17:15 **Satellite Symposium on Bionics**
Introduction:
Prof. Tamás Roska
Pázmány Péter Catholic University, Budapest
Bionics – An Emerging Discipline and High Technology

17:15–18:00 **Plenary lecture V.**
Prof. Ronald Tetzlaff
University of Dresden
Implantable Systems: Seizure Prediction in Epilepsy?

19:00 **Dinner, Domonvölgy Lázár Equestrian Park**

15 June, Saturday

09:00–09:30 **Satellite Symposium**
Prof. Sabine van Huffel
Catholic University of Leuven
Advances in Neonatal Brain Monitoring

09:30–10:00 *Prof. Gianluca Ciardelli*
Technical University of Torino
Materials Design at the Nanoscale for Biomedicine and Bionics

10:00–10:30 *Prof. Danilo DeMarchi*
Technical University of Torino
Nanogap Based Electrodes for the Study of Single Bio-molecules

10:30–11:00 *Prof. Emmanuel Drakakis*
Imperial College, London
Harnessing the MOS Transistor Non-linearity: From Bionic Ears to Cytomimetic Circuits

11:00–11:20 **Coffee break**

11:20–11:50 *Prof. Fernando Corinto*
Technical University of Torino
Memristor technology in Neuro-Bio-Inspired Systems

11:50–12:05 *Prof. Péter Mátyus*
Semmelweis University, Budapest
Péter Pázmány Catholic University, Budapest
New Perspectives in Bionics: Innovative Breakthroughs on the Horizon

12:05–13:00 **Round Table Discussion**
Curricula with Disciplinary Pillars from Electronics, Computing and Life Sciences

13:00–13:10 **Closing remarks**

13:10–14:00 **Lunch**

INVITED PRESENTATIONS

Computational Methods in Drug Discovery: Diffusion in the Scientific Community and Successful Experiences

Stefano Alcaro
Laboratorio di Chimica Farmaceutica del Dipartimento di Scienze della Salute, Università "Magna Græcia" di Catanzaro, viale Europa, Catanzaro, (Italy)

The development of new drugs is a challenging goal of any medicinal chemist working in academia or in pharmaceutical companies. In this context, integration of computational techniques in the discovery environment is expected to speed up the process with relatively low expenses. Today the structural biology, especially by means of the Protein Data Bank [1] and other web bases resources, communicates directly with the medicinal chemistry, allowing to perform the rational drug design of novel compounds in a modern fashion.

In order to look for the diffusion of such techniques in the scientific community a detailed questionnaire has been distributed to specialists belonging to academia and pharmaceutical industries during a meeting for computational chemists carried out in Italy in 2011. The results of the analysis attested that among 18 different computational tools (Figure 1) three of them are ranked with high levels of potential and real interests [2].

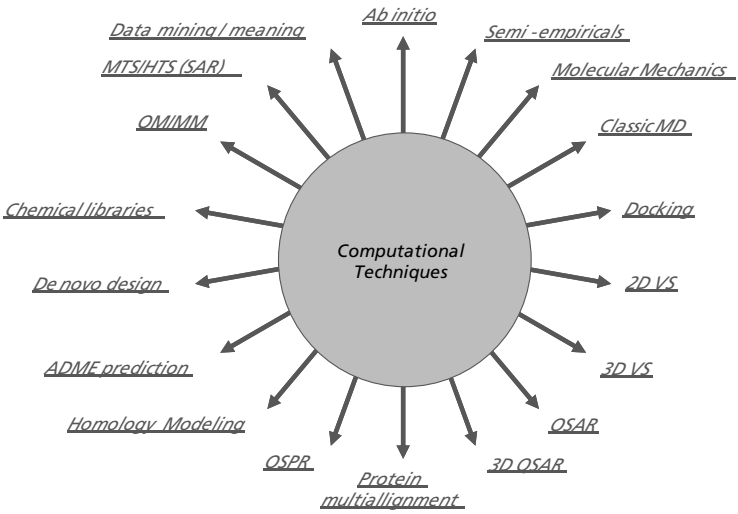


Figure 1: list of the computational tools investigated by the survey.

In this communication a brief discussion about the computational most trusted methods will be carried out and some successful stories will be presented in order to give an idea about their real impact and advantage for the scientific community.

Finally, some considerations will be devoted to define the formative goals of high level of education courses with the aim to enrich the competencies of experts in this field adequate to perform drug discovery programs in academia as well as in biotech and pharmaceutical companies.

This research work is supported by the Italian Ministry of Education FIRB_IDEAS (code RBID082ATK) and PRIN 2009 (code 2009MFRKZ8).

References

1. Bourne, P.E. et al Brief Bioinform. 2004, 5, 23-30
2. Alcaro, S. et al Future Med Chem. 2013, asap

Materials design at the nanoscale for biomedicine and bionics

Gianluca Ciardelli
Department of Mechanical and Aerospace Engineering, Politecnico di Torino, Turin, Italy

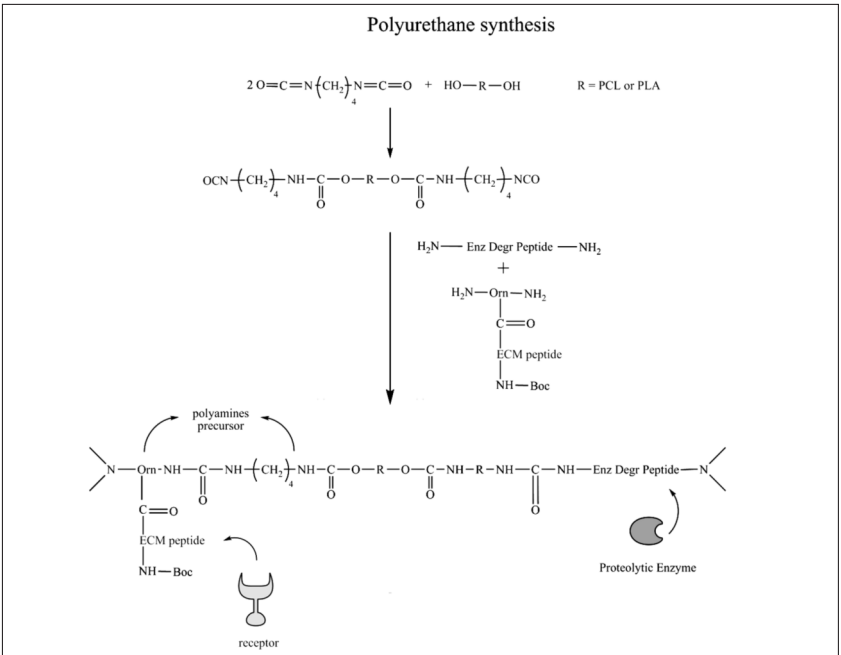
Demand for medical implants is estimated to increase 9.3% annually to US$43.6 bln in 2011, according to a report by Freedonia Group. Cardiac implants are expected to remain the top-selling group, led by stents and defibrillators, with demand expanding 9% pa to almost US$20 bln in 2011. Biodegradable plastics, that have found until now application in packaging, are slowly capturing newer markets, particularly medical products. Applications of biodegradable plastics have gained market acceptance in the medical sector in: medical implants, drug delivery, hernia repair devices. One reason for the rise in implant use is the performance and outcome advantages over alternative treatments, such as drugs. Another reason is the constant improvement and innovation of devices that keep getting smaller.

However, materials which were approved (e.g. polymers belonging to the polylactic or polyglycolic acids family) were originally designed for other applications and then proposed for medical use. This approach usually results in various drawbacks in the final application, e.g. the release of acidic compounds during the degradation of polylactic acid results in a lowering of the local pH at the implant site, with consequent danger of inflammation reaction and adverse body response. Consequently, it is clear that a precise design at the nanoscale of the chemical and morphological structure, can open the way to a new generation of biomaterials tailor-made to the challenging applications of

biomedicine and bionics, such as tissue regeneration, advanced diagnostics, cancer treatment.

In this context, this contribution will present the more recent research results of the Biomedical Laboratory at Politecnico di Torino on the application of proprietary, degradable block copolymers in the regeneration of the cardiac, nervous and bone tissue and in nanomedicine.

The rationale for polymer design illustrated in the figure below, where the different building blocks are used to provide the final product with the expected mechanical properties during use, cell-adhesion and -targeting motifs, hydrolytic and enzymatically activated degradation (enhanced in the presence of pathological conditions), providing non toxic and bioactive degradation products. The polymers can be processed then in the suitable form (tubular structures, anisoptropic scaffolds, nanoparticles, injectable gels) for the final application.



POLYURETHANES SEGMENTS

Blue. Polyamines precursor: Ornithine (Orn) and Putrescine (Butane diisocyanate derivatives). Polyamines are able to regulate tissue regeneration.

Red. Enzymatic Degradable Peptide (Enz Degr Peptide). Peptide sensitive to enzymatic activity.

Green. Peptide belongin to a protein of Extracellular Matrix (ECM peptide). Cell binding domain (e.g. RGD, REDV, IKVAV).

Purple. Macrodiol: poly(lactic acid) diol (PLA diol) or polycaprolactone diol (PCL diol). Cristalizable and degradable block.

Memristor technology in Neuro-Bio-Inspired Systems

Fernando Corinto
Technical University of Torino

Biological neural systems use self-reconfigurable and self-learning primitive elements (synapses) to extract relevant information from complex and noisy environments, to detect specific spatio-temporal patterns in the data of interest and to compute and simultaneously store some significant features. All these desirable attributes may be realized by using two-terminal elements, memristors (memory resistors) which most closely resemble biological synapses.

This talk presents a comprehensive nonlinear circuit-theoretic foundation for novel circuit implementation of the Hodgkin–Huxley neural model with memristors.

Nanogap based electrodes for the study of single bio-molecules

Danilo DeMarchi
Technical University of Torino

The study of molecules at level of single or of few molecule clusters is opening new horizons in terms of quality of the analysis, biosensor resolution, and by sure novel characteristics will be discovered working at single molecule dimension. The lecture presents several experiments in which different types of proteins are analysed. The study exploits the capabilities of a custom platform, the Nanocube, ready for the stimulation and management of the signals of the bio-molecules, and for their elaboration. Proteins as Reaction Centers and Bacteriorhodopsins have been used for realising the Metal-Molecule-Metal junctions, the basic structure for the implementation of the bio-nanodevices. In this work he possibility to realise bioelectronic elements with sizes at the nanometric scale was demonstrated.

Harnessing the MOS Transistor Non-linearity: From Bionic Ears to Cytomimetic Circuits

Emmanuel Drakakis
Imperial College, London

The physics-dictated sub-threshold operation of MOS transistors can lead to ultra-low-power designs but is also governed by a challenging exponential characteristic. This talk will elucidate how to view such a non-linearity as an asset and how to exploit it by treating it as a powerful computational primitive which can lead to the systematic realisation of: a) continuous-time continuous-value Externally Linear and time-invariant but Internally Non-linear (ELIN) biomimetic behaviours, and b)non-linear dynamics dictated by biology. High-performance ELIN realisations matching the high-dynamic range and mimicking the operation of the basilar membrane of the inner ear will be presented and their role in low-power Bionic Ear processors will be highlighted. Several examples of systematic computation of non-linear cellular and molecular dynamics by means of ultra-low-power cytomimetic circuits will also be elaborated.

How can pain and the effect of new analgesics be investigated in animal models?

Zsuzsanna Helyes
University of Pécs, School of Medicine, Department of Pharmacology and Pharmacotherapy & János Szentágothai Research Centre, Molecular Pharmacology Research Team, Pécs, Hungary

Conventional analgesics are not effective in several chronic persistent pain, the therapy is often not satisfactory. Unfortunately, breakthroughs have not been achieved in the last century in this field. Therefore, appropriate models and complex investigational techniques are particularly important to elucidate the pathophysiological mechanisms and identify novel drug targets. Pain is difficult to investigate in animals: only nocifensive behaviours can be determined as functional parameters, and certain molecules of interest can be examined with biochemical/immunohistochemical analysis in the pain pathway. Traumatic mononeuropathy can be induced by unilateral partial tight or total loose ligation of the sciatic nerve or the lumbar L4-L5 dorsal roots. The first one is technically easy, perfectly reproducible and reliable, there is no motor disorder. Their major advantage is that self-control comparisons can be done with the intact contralateral side. Cytostatics (cisplatin, paxitaxel) induce toxic, and the pancreatic β cell-destroying streptozotocin evoke diabetic

(metabolic) chronic polyneuropathy, but it is accomplanied by systemic toxicity, nephropathy and weight loss, which influence the functional responses. The characteristic symptoms are hyperalgesia (decrease of the pain threshold) and allodynia (non-painful stimulus becoming painful). These can be determined by measuring touch sensitivity of the paws by dynamic plantar aesthesiometry and pressure thresholds by analgesimetry. Heat sensitivity does not change in neuropathy despite inflammation, but cold tolerance determined by the withdrawal latency from icy water remarkably decreases. Spontaneous motility is examined in the open field test, motor coordination on a Rota-Rod wheel. Neuronal and glia activation markers in the peripheral nerve, dorsal root ganglia, dorsal horn, periaqueductal grey, thalamus and somatosensory cortex can be detected, quantified and correlated with the functional results. The expression of certain molecules can be followed by *in vivo* imaging in chronic models. We have provided evidence that targeting peripheral sensory nerve terminals with special emphasis on somatostatin sst_4 receptors and Transient Receptor Potential ion channels is promising to develop novel analgesics.

For potential risks and side-effects please contact with a zebrafish

Urbanyi B., Bakos K., Kovacs B., Kovacs R. and Csenki Zs.
Szent István University, Faculty of Agricultural and Environmental Sciences,
Department of Aquaculture

Among millions of species only a few could „make a career" and reach the same popularity as the tiny zebrafish *(Danio rerio).* This success is mainly due to its decorative appearance and easy husbandry, so its popularity has been undiminished since the discovery of the species (early 19th century). However its scientific carrier began a bit later, in the middle of the 1970s when the Hungarian-born researcher, George Streisinger started to use the species as a laboratory model. Zebrafish possesses a number of advantages for scientists too including small size, broad tolerance and rapid ontogenesis. Embryos are transparent and develop *ex utero* allowing the monitoring of tissue and organ development *in vivo* and embryogenesis can easily be followed through the transparent chorion. Initially, the species was mainly used as a model for developmental biology but thanks to the advances in science, especially in molecular biology and biotechnology, zebrafish became popular in many other fields of science. Nowadays, researchers conduct toxicology, biomedical, tumor biology and behavioral studies on zebrafish, use the species as a cardiovascular or neurological disease model in over 900 laboratories. A series of OECD test guidelines and protocols recommend zebrafish as a test species.

Moreover, zebrafish might also be „asked" about the main mechanisms of action or the side effects of medicinal substances. Fish are able to take up substances from the medium even in relatively low concentrations and respond similarly to mammals. Besides these, full genome sequence and the availability of a wide range of genome manipulation techniques make the zebrafish model surpass mammalian models in some experimental designs.

The Department of Aquaculture (Institute of Environmental and Landscape Management, Faculty of Agricultural an Environmental Sciences) at Szent István University has been using zebrafish for at least 7 years. Recently, mainly toxicological studies of substances including drugs or potential drug candidates are conducted. Besides classical toxicity testing, cardiovascular (ECG) and molecular biological tests are carried out. According to the increasingly stringent regulations, transgenic biomarker lines are also developed as new alternatives to mammalian models, allowing the *in vivo* monitoring and modelling of certain physiological or molecular disorders.

In the presentation we introduce the zebrafish as a model organism, discuss its use in medical and toxicology research and highlight some important results and achievements of Hungarian researchers and the Department of Aquaculture.

Advances in Neonatal Brain Monitoring

S. Van Huffel[1,2]
[1]ESAT-SCD(SISTA), Dept of Electrical Engineering, KU Leuven, Belgium
[2]iMinds Future Health Department, Leuven, Belgium

Why monitoring brain function in the newborn baby?

The neonatal brain is vulnerable to various insults occurring in the perinatal period. These include a temporary lack of oxygen and blood supply (asphyxia), metabolic disturbances (disturbances in blood glucose, calcium, sodium etc.), infections, stroke and trauma. Often the neonatal brain reacts to these injuries by suppression of the electrical activity of the brain cells (neurons) and by expressing seizures. A premature brain has similar morphologies, for which an evolution towards a normal term brain is wished. Monitoring neonatal brain activity helps in detecting these harmful conditions and the evolution in an early and potentially reversible stage, allowing timely treatment. This will help to prevent serious damage to the brain.

Automated methods of neonatal brain monitoring

There is an unmet need for reliable methods for automated brain monitoring in neonates admitted to the Neonatal Intensive Care Unit (NICU) at risk for brain damage.

Several measurement technologies, such as Electro-EncephaloGraphy (EEG), Cerebral Function Monitoring (CFM) and Near-Infrared Spectroscopy (NIRS) , are used to measure brain activity. Due to time constraints, the talk is restricted to recent advances in EEG based automated brain monitoring and to the detection of two pathologies: hypoxic insults and seizures.

EEG analysis

Various research groups have published neonatal seizure detection methods in the last few years. However, there is no validated system that is reliable and ready for use at the bed-side. Our research group has developed a reliable automated seizure detection system, which mimics a clinical neurophysiologist reading the EEG. In essence, seizures are classified according to their main morphology, namely spike-trains and oscillations, or combinations of both. Algorithms have been developed to detect these patterns and have been further refined, mainly by means of automated artifact reduction using blind source separation. Additionally, tensor algorithms have been developed for seizure onset localization. The results are displayed as topographic maps. This software module is part of our our neonatal brain monitor, named NeoGuard, and detects seizures with a sensitivity of 85% to 90% and false positive rate of 0.1 to 0.4 per hour, depending on EEG background severity, and is suited for clinical use. Recent advances include automation of EEG background analysis, mainly with the goal of classifying the severity of encephalopathy in neonates and detecting dynamic changes (improvement or deterioration in the background activity). Bursts and interburst intervals in the EEG background have been shown in previous studies to be the most sensitive parameter for neurological outcome prediction in asphyxiated neonates. Novel ways for automated EEG background grading are presented. In particular, this automated background classification should enable to refine the selection of neonates with moderate to severe postasphyxial brain dysfunction (hypoxic ischemic encephalopathy) for therapeutic hypothermia. Finally, suggestions for future developments in monitoring and algorithm development are given.

POSTER PRESENTATIONS – MEDICINE

Urinary flow studies of young boys after hypospadias repair

Dóra Balázs, Zsuzsanna Antal
1st Department of Pediatrics, Semmelweis University, Budapest, Hungary

Introduction: Hypospadias is one of the most common congenital anomaly affecting boys. As treatment, there are countless types of accepted surgical techniques. To compare the results of these solutions the most widely used method is uroflowmetry. The aim of the present study is to identify urethral stricture among patients who underwent hypospadias repair, recognizing the impact of this complication on the bladder and the kidney function, and to compare our results to the international literature.

Methods: A total of 41 children, who had undergone urethral reconstruction surgery at the 1st Department of Pediatrics of Semmelweis University between 2005 and 2008 were examined. Beside uroflowmetry patients' history, physical examinations and abdominal ultrasonography were performed. The measured urine flow rates were evaluated with the hungarian Miskolc nomogram as well as with the internationally spread Toguri nomogram. The patients were separated in 3 groups according to the severity of the hypospadias and to the type of the formerly applied surgery: I. glandular – MAGPI; II. distal – Snodgrass; III. proximal – Duckett and Hadidi.

Results: The average age of the children was 7.3 years; the average time passed between the surgery and the examination was 4.6 years. Both the fistula formation and the stenosis were significantly more frequent in group III. Although, only 7% of all children complained about weak urine flow, 38% had a flow under 5 percentil for first sensation, and with full bladder this number reaches 67.5%. The exact values are: 20% and 40% in group I., 41% and 67% in group II., and 60% and 70% in group III., respectively. These rates are slightly higher than the data found in the literature. The number of children with urine flow under 5 percentil was significantly lower on the Miskolc nomogram than on the Toguri nomogram (49% and 19% respectively). Three children in group II. (11%) and six children in group III. (60%) had residuum after voiding.

Conclusion: Abnormal uroflow parameters, referring to stenosis are very common among symptom-free children after hypospadias surgery. The cause of the variant incidence of stenosis described in the literature may be the use of different nomograms. Determining the long-term consequences requires careful follow-up.

Exhaled biomarker pattern is altered in children with obstructive sleep apnoea syndrome

Benedek Pálma[1], Zsófia Lázár[2], András Bikov[2], László Kunos[2], Gábor Katona[1], Ildikó Horváth[2]
[1]Heim Pál Children Hospital, Budapest, Hungary
[2]Semmelweis University, Budapest, Hungary

Objectives: Obstructive sleep apnoea syndrome (OSAS) is a common disorder in children, which is associated with enhanced inflammatory status. Inflammation-associated changes could be monitored by the assessment of exhaled biomarker profile. This study aimed to compare the exhaled biomarker profile in children with OSAS and habitual snorers.

Methods: Eighteen children with OSAS (8 ± 2 yrs, mean ± SD) and ten non-OSAS subjects with habitual snoring (9 ± 2 yrs) were recruited. Exhaled breath was collected from the lower airways, processed using an electronic nose and analyzed off-line using principal component analysis, followed by discrimination analysis and logistic regression to build a receiver operating characteristic (ROC) curve.

Results: Exhaled biomarker pattern of OSAS patients was discriminated from that of control subjects (p=0.03, cross-validation accuracy: 64%), ROC curve analysis (area: 0.83) showed 78% sensitivity and 70% specificity.

Conclusions: The altered exhaled biomarker pattern in OSAS might reflect accelerated airway and/or systemic inflammation in diseased state. Breath pattern analysis by an electronic nose can serve as a new tool to monitor inflammation in children with OSAS.

Evaluation of immunoactivity with nuclear medicine methods in orbits of patients with graves' disease without endocrine orbitopathy

Eszter Berta[1,2], Miklós Bodor[1,2], Lajos Szabados[3], László Galuska[3], Annamária Erdei[1], Annamária Gazdag[1], Bernadett Ujhelyi[4], Endre V. Nagy[1]
[1]Department of Internal Medicine, Division of Endocrinology; [2]Division of Clinical Pharmacology; [3]Center of Nuclear Medicine; [4]Department of Ophthalmology, Medical and Health Science Center, University of Debrecen, Debrecen, Hungary

Background: Graves' ophthalmopathy (GO) is a common and severe complication of Graves' disease (GD), furthermore high percentage of cases are responding poorly to therapy. The involvements of the orbits are not always present at the beginning of GD. According to our present knowledge development of eye involvement cannot be

predicted. Our goal was to find evidence that orbital autoimmune activity, being the first stage of GO, is predictable using orbital DTPA SPECT. Besides, we aimed to answer the intriguing question whether orbital activity can be identified in patients who do not develop a clinically present GO during their follow-ups.

Methods: fifty-four orbits of twenty-seven patients newly diagnosed with GD were entered into the study. All patients underwent an ophthalmological examination; individuals showing signs of present GO were excluded from the study. GO was classified according to the Clinical Activity Score. None of the patients had received antithyroid drugs or ophthalmic measures while entering the study. A ^{99m}Tc-labelled diethylene-triamine pentaacetic acid (^{99m}Tc-DTPA) scintigraphy was performed at the beginning, during the follow-up period when endocrine ophthalmopathy was observed or eventually at the end of the follow-up period, after one year. The results were compared with data obtained from 34 orbits of 17 patients with Raynaud's phenomenon who underwent DTPA SPECT examination for diagnostic reasons. None of the individuals in the control group had thyroidal disease.

Results: During the one-year long follow-up time 6 out of the total of 27 patients (22%) were discovered with newly developed GO. The mean DTPA uptake of the orbits of Graves' patients with or without later GO was significantly higher than the results obtained in the control group (10.45±1.72 MBq/cm^3, 9.18±1.18 MBq/cm^3 and 7.7±2.44 MBq/cm^3, respectively, $p<0.05$).

Results: Our findings showed that the patients with Graves' disease with or without a later developing GO had a significant retrobulbar activity according to scintigraphic imagination in comparison to the normal control group. Our results also revealed that an ongoing subclinical inflammation can be detected using DTPA spectography examination method regardless of the clinical activity classified with CAS.

Complex investigation of the role of capsaicin-sensitive sensory nerves in a mouse polyarthritis model

Bálint Botz[1,2], Éva Borbély[1,2], Kata Bölcskei[1,2], Tibor Kenyér[2], Péter Nagy[1,2], Tamás Németh[3], Miklós Kovács[3], Attila Mócsai[3], János Szolcsányi[2], Zsuzsanna Helyes[1,2]
[1]University of Pécs, School of Medicine, Department of Pharmacology and Pharmacotherapy, Pécs, Hungary; [2]University of Pécs, János Szentágothai Research Centre, Molecular Pharmacology Research Team, Pécs, Hungary; [3]Semmelweis University, Department of Physiology, Budapest, Hungary

Capsaicin-sensitive sensory nerves have complex regulatory functions in the joints under physiological and pathophysiological conditions depending on the mechanisms and the involvement of the simultaneously released pro- and anti-inflammatory

neuropeptides. In the present study we determined their role in a mouse model of rheumatoid arthritis by functional, metabolic and structural examinations.

Peptidergic nerves were defunctionalized by the capsaicin analog resiniferatoxin in C57Bl/6 mice, intact animals served as controls. Arthritis was induced by KxBN arthritogenic serum, and the inflammatory, motor and nociceptive characteristics of the disease were monitored for 14 days. Matrix metalloproteinase (MMP) and myeloperoxidase (MPO) activities in the joints were assessed by *in vivo* fluorescent molecular tomography and luminescence imaging. Periarticular bone morphology was investigated and quantified by self-control micro-CT scans.

Edema of the tibiotarsal joints and motor impairment in the wire grid test were significantly enhanced, but pain-related mechanical hyperalgesia and periarticular bone destruction were diminished in mice pretreated with resiniferatoxin compared to the non-pretreated ones. Both MMP and MPO activities were considerably greater in arthritic joints, the latter being significantly higher in desensitized animals.

Activation of peptidergic sensory nerves modulates arthritis-related alterations in a complex manner: it induces pain and promotes bone loss, but prevents swelling, MPO activity increase and grasping failure. MMP and MPO enzymes are both important inflammatory factors, but the activation of MMP is not regulated by these fibres.

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Signal Processing for Monitoring Cerebral Hemodynamics in Neonates

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Changes in cerebral hemodynamics (CH), especially changes in cerebral blood flow (CBF), might cause brain damage due to ischemia or hemorrhage, which can lead to motor and developmental disabilities. Premature infants are susceptible to changes in these mechanisms. Hence, monitoring CH in this population is of vital importance in order to prevent brain damage.

Traditionally, CH has been investigated by the use of linear methods including measurements of mean arterial blood pressure (MABP), concentration of CO_2, and CBF. Since the relation between MABP, CO_2 and CBF is nonlinear, nonlinear methodologies are more suited to address this problem. But, nonlinear methods are difficult to interpret clinically. In addition, measurements of CBF are difficult to obtain in premature infants.

Near-infrared spectroscopy is a noninvasive technology that allows the monitoring of cerebral oxygenation, which, under certain conditions, can be used as a surrogate measured for CBF. However, NIRS presents several drawbacks that hinder its clinical application. One of its main drawbacks is that it is influenced by third signals, such as variations in arterial oxygen saturation SaO_2.

In this study we present the use of subspace projections as a preprocessing step for NIRS measurements, where the influence of SaO_2 is subtracted from the NIRS signals. In addition, we present the use of a novel, clinically interpretable, nonlinear model for CH monitoring, based on kernel principal component regression and subspace projections. This model is able to retrieve the nonlinear contribution of MABP and CO_2 from the NIRS measurements.

The methodologies presented in this study provide a robust and adequate framework for the monitoring of cerebral hemodynamics in neonates.

Pharmacokinetics and pharmacogenomics of high-dose methotrexate treatments in pediatroc ALL

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High-dose methotrexate (HD-MTX) is an important component of the consolidation therapy of childhood acute lymphoblastic leukemia (ALL).

Aim: Our objectives were to perform a detailed comparative study of pharmacokinetics and toxicity of methotrexate (MTX) and 7-hydroxy-methotrexate (7-OH-MTX) after HD-MTX treatments and to analyze their relation with common and rare polymorphisms in genes of folate metabolic pathway, transporter molecules and transcription proteins.

Methods: Data of 65 children treated with 5 g/m^2/24h and 88 children treated with 2 g/m^2/24h HD-MTX according to ALL-BFM 95 and ALL IC-BFM 2002 protocols were collected [mean age: 6.4 years (1.0-17.9 years)]. Totally 583 HD-MTX infusions were analyzed. Hepato-, nephro- and bone marrow toxicities were evaluated. 63 single nucleotide polymorphisms (SNP) of 14 genes were genotyped. Random forest and regression trees were used for variable selection. Linear mixed models were established to prove the significance of the selected variables and to estimate effect sizes.

Results: 5 g/m^2/24h treatments resulted in higher serum and CSF MTX and 7 OH MTX

levels (p<0.001). CSF penetration rate of MTX was independent of given dose [0.023 (95% CI: 0.017-0.025) vs. 0.028 (95% CI: 0.024-0.03)]. CSF MTX concentration correlated with 24h serum MTX level. Slightly more but reversible side effects were seen after 5 g/m^2/24h MTX. SNPs (rs4948502, rs4948496, rs4948487) of ARID5B gene were associated with serum levels of MTX (p<0.001), serum levels and AUC of 7-OH-MTX (p<0.001) and with hypoproteinaemia (p<0.001). The rs4149056 of SLCO1B1 showed also significant association with the serum levels of MTX (p<0.001).

Conclusions: Therapeutic serum and CSF MTX concentrations can be achieved more reliably with 5 g/m^2/24h treatments. We confirmed the association of ARID5B gene and MTX plasma levels however the exact role of this gene on MTX levels needs further investigations.

VGluT3-containing raphe neurons represent a new modulatory possibility

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Nucleus raphe medianus (MR) provides a strong serotonergic input to the hippocampus, whereas almost half of the projecting MR cells including some serotonergic ones express the vesicular glutamate transporter VGluT3. Previously, we reported VGluT3-containing MR-fibers as forming synapses on hippocampal interneurons. Stimulation of this pathway resulted in fast, temporally focused activation of postsynaptic cells, mediated by ionotropic glutamate receptors. This effect raises the possibility of a novel type of modulatory control within the raphe-hippocampal system, as well as the question whether VGluT3-containing cells form a distinct subgroup of MR neurons.

Aims: We registered the firing of MR neurons and hippocampal activity simultaneously in urethane-anesthetized Wistar rats. Recorded cells were labeled with Neurobiotin for further anatomical identification; serotonin- and VGluT3-content were also determined. Dendritic arbor of some neurons was reconstructed as well. In electrophysiological analysis, we focused on changes of firing behavior during hippocampal state transitions.

Results: Recorded and identified cells were grouped into four subpopulations: serotonin-immunopositive, VGluT3-immunopositive, double positive and double negative cells. Serotonergic neurons were slow firing regardless of VGluT3-expression. In contrast, non-serotonergic VGluT3-immunopositive cells fired significantly faster and in a more complex manner than the serotonin-containing populations indicated by high interspike interval variability. Importantly, the firing pattern of VGluT3-immunopositive cells changed more significantly during hippocampal state transitions compared to the serotonergic

subgroups. Morphological analysis unraveled profusely branching dendritic tree with shorter dendrites of serotonergic cells whereas the dendrites of both VGluT3-immunopositive and double positive neurons were longer with minimal number of branches. Taking together, VGluT3-containing neurons form a distinct subpopulation in the MR potentially contributing to state transitions.

Clinical utility of genetic testing in adolescent onset nephrotic syndrome – case report

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Objectives of study: Approximately 10% of children and 40% of adults with idiopathic nephritic syndrome (NS) are steroid resistant (SRNS) and progress to end-stage renal disease (ESRD) requiring dialysis or renal transplantation. In these cases, renal histology typically shows focal segmental glomerulosclerosis (FSGS). Mutations in the genes NPHS1, NPHS2, CD2AP, ACTN4, WT-1, located on different chromosomes, expressed by glomerular podocytes have been identified in patients with SRNS. Immunosuppressive medication is not helpful in the genetic forms of NS, and kidney transplantation is the only curative therapy.

Material and methods: The authors report the case of a 13 year old girl, who presents to the 2nd Pediatric Clinics from Târgu-Mureş with generalized edema, laboratory data revealing nephritic-range proteinuria (7.18 g/die), hypoproteinaemia (41 g/l), hyperlipidaemia. The evolution under steroid treatment was unfavorable with persistent massive proteinuria (7.18 g/die), secondary arterial hypertension (BP: 140/90 mmHg) and transient hyperglycemia. Renal biopsy was performed, histopathology showing FSGS. Genetic testing revealed mutation in the gene WT1. ESRD develops 9 months after the onset of the illness. After kidney transplantation the evolution was favorable, in this moment the patient is in complete remission.

Conclusions: WT1 gene mutation is often identified in NS and in this form of NS does not respond to immunosuppressive therapy and progress rapidly to ESRD, but after kidney transplantation relapse is not expected. The early genetic diagnosis in SRNS is time-consuming and expensive, but it is important for proper clinical management of the patients, prognosis and genetic counseling of the families.

Improving spike classification in neural and surface electromyography (sEMG) data

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The term "spike" is used to describe a short-time event that is the result of the activity of its source. Spikes can be seen in different signal modalities. In these modalities, often more than one source generates spikes. Classification algorithms can be used to group similar spikes, ideally spikes from the same source. This work examines the classification of spikes generated from neurons and muscles. When each detected spike is assigned to its source, the spike trains of these sources can provide information on complex brain network functioning, muscle disorders, and other applications. During the past several decades, there were many attempts to create and improve spike classification algorithms. No matter how advanced these methods are today, errors in classification cannot be avoided. Therefore, methods that would determine and improve reliability of classification are very desirable. In this work, it is attempted to find efficient solutions for post-processing of spike trains extracted after the classification to increase their accuracy and reliability. This is done for two different signal types: neural and muscle signals. The aim was to create a reliable and automated signal processing framework. In modern neural microarray probes, many recording channels provide large quantities of data, which require an enormous amount of time to be analyzed. A fully automated system is therefore needed but is still out of reach. As for the analysis of muscle signals, treated by surface electromyography (sEMG), many recording channels observe the same spikes simultaneously. Due to the frequently overlapping spikes, only automated sophisticated methods are able to perform classification.

Functional polymorphism C385A of the Fatty Acid Amide Hydrolase gene is not a genetic susceptibility factor for polycystic ovary syndrome

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Objective: Polycystic ovary syndrome (PCOS) is the most common endocrine disorder among women of reproductive age. Overactivation of the endocannabinoid system contributes to dyslipidaemia, insulin resistance and obesity, all of which are comorbidities

related to PCOS. The enzyme fatty acid amide hydrolase (FAAH) is responsible for the degradation of anandamide, a key messenger of the endocannabinoid system. C385A functional polymorphism of the gene encoding FAAH has been associated with reward disorders, such as overweight and obesity. Our aim was to investigate the possible association of C385A polymorphism with PCOS.

Patients and Methods: Toward this aim we designed a monocentric pilot study. 130 Caucasian women of reproductive age were enrolled, 63 of which were diagnosed with PCOS according to the Rotterdam consensus criteria. Anthropometric and laboratory parameters were acquired from subjects. The alleles of the polymorphism were detected using polymerase chain reaction (PCR) and subsequent cleavage by Eco130I (StyI) restriction endonuclease. Appropriate diagnosis of the PCR was confirmed by direct DNA sequencing. For statistical analysis Mann-Whitney test and $\chi 2$ test were used.

Results: No difference was found in minor allele frequency between patient and control groups. Patients bearing the mutant allele had higher free thyroxine levels. Healthy control subjects carrying the polymorphism had lower insulin levels.

Discussion: Upon our results we can conclude that C385A functional polymorphism is not a genetic susceptibility factor for PCOS. However, the polymorphism might have a role in influencing the synthesis or metabolism of certain hormones, such as thyroxine and insulin. Statement of contribution PR and KR designed the experiments. PR, TF and GS diagnosed patients. VKG, BS and PR performed laboratory research. VKG, AP, PR performed statistical analysis.

A new methodical innovation approach: Three dimensional histological examination of dental root cement

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Motivation and objectives: The goal of our team was the development of „true three-dimensional (3D) transparent intravital microscopy". At first, we established the basis of real 3D imaging in microscopy and constructed a prototype of a 3D vital microscope. Recently, we have been dealing with the improvement of clearing solutions, that were able to make the soft tissues (e.g.: liver, small intestine, salivary gland) transparent. In our recent experiment we intended to investigate the clearing effect of these materials on hard tissues, specially teeth.

Methods: We prepared cross sectional and vertical slices of extracted human teeth and investigated the clearing effect of one of our newly developed physiologic solution. For the inspection we applied the above mentioned 3D vital microscopy. We documented our observations by means of 3D photographs.

Results: The newly developed solution was able to clear the hard dental structures. Acellular cement has rather granular structure; meanwhile the cellular cement looks like to have lamellar structure. In the cellular cement, we were able to detect coal black, dendritic cells, without the usage of any histochemical staining. It was quite frequent, that we detected such form of cell groups, where there was a centrally oriented pyramid-like cementocyte, encircled by cells in wreath. The processes of these latter cells contacted with each other, creating a web like impression. We had the opportunity to observe some typical cell forms just like pyramid, roundabout, polyhedron, ovoid and cylindrical. At the end of the processes we quite often recognized butt-like flares or rod-like ramifications. In case of the cellular cement, the periodontal fibers anchored in between the superficial crests, meanwhile by the acellular cement they could pass a little deeper among the granules.

Conclusion: Our newly developed system is suitable to clear and investigate not only the soft tissues, but the more challenging hard tooth structures as well, promoting real 3D qualitative and quantitative histological observations, which are of great importance regarding the functional and clinical consequences.

The effects of angiotensin II on the NMDA-type glutamate receptors of the pyramidal cells in the prefrontal cortex

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The prefrontal cortex (PFC) and its pyramidal cells have an important role in learning and behavior. Angiotensin II (AT) influenced learning and memory in behavioral tests. Our aim was to study the effects of AT on NMDA receptors of pyramidal cells in PFC with patch clamp. Whole-cell voltage-clamp access was established in pyramidal cells of brain slices prepared from 10-11 days old rats. The cells were perfused with artificial cerebrospinal fluid (ACSF) (2,5-3ml). NMDA (30 μM) applied 3 times for 1.5 min with 10-min intervals between applications, induced inward currents (T1-3). Effects at T3 were presented as T3/T2. More than ±15% change at T3 compared to T2 was considered as an effect. For statistical analysis we used one-way ANOVA and Bonferroni correction ($p<0,05$). AT was given 5 minutes before and during T3, AT1 receptor antagonist eprosartan (1μM) was in the ACSF all along. 0,3μM AT enhanced NMDA currents in a

subpopulation of the cells, in the rest there were no effects. 1μM AT enhanced the currents in 50% of the cells, inhibited them in 25%, in the rest it was ineffective. Eprosartan reversed the enhancement in both concentrations. 3μM AT either inhibited the currents or it was ineffective. Eprosartan did not influence these effects. Thus AT has dual effect: 0,3-1μM AT enhances the currents in a subpopulation of the cells, which are reversed by eprosartan. These currents are probably AT1 receptor mediated. 1-3μM AT reduces the currents in another subpopulation, which are not influenced by eprosartan.

The contribution of matrix substrate-level phosphorylation to superstoichiometric P/O ratios of isolated mitochondria

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The Phosphate/Oxygen ratio (P/O ratio) is the number of ATP molecules synthesized by oxidative phosphorylation for each pair of electrons passing via the respiratory chain. The P/O ratio is a core metabolic parameter, but is notoriously difficult to measure. This is because determining ATP made by suspensions of mitochondria is error prone; mitochondria might be damaged; furthermore, the P/O ratio depends on the structure of the FoF1 ATP synthase. Finally, ATP effluxed from mitochondria may also derive from substrate-level phosphorylation taking place in the matrix. We tested the hypothesis that ATP emerging from matrix substrate-level phosphorylation influences the P/O ratio of isolated mitochondria, and if higher than expected P/O ratios can be recorded by substrates generating succynil-CoA. Mouse liver mitochondria from C57B1/6 mice were prepared by standard differential centrifugation protocols. Oxygen consumption and ADP-ATP flux rates were measured in parallel from the same mitochondrial sample using O2k Oxygraph-fluorimeter. Mitochondria were energized by substrates and their combinations that favour substrate-level phosphorylation (glutamate, alpha-ketoglutarate, malate), versus those that do not (succinate, fumarate). Experiments were replicated 8 times, on four independent occasions. Glutamate, alpha-ketoglutarate, malate and their combinations yielded higher P/O ratios than succinate and fumarate. Our results support the notion that those substrates yielding a higher flux of succinyl-CoA in the mitochondrial matrix, result in 'superstoichiometric' P/O ratio determinations, and should be taken into account when extrapolating the amount of ATP produced exclusively by the FoF1 ATP synthase.

Identifying and testing compounds selectively toxic to multidrug resistant cancer cells

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Treating malignant tumors by chemotherapeutics often results in the emergence of drug resistant cancer. Resistant tumor cells may become protected against other cytostatics with similar mechanism of action, thus forming the so-called multidrug resistant (MDR) phenotype. One of the main reasons of MDR is the overexpression of ABC transporters. These transporters are able to efflux xenobiotics from the cells. Among ABC transporters, the most common and most relevant resistance is conferred by ABCB1 (P-gp), which is able to recognize numerous chemotherapeutics currently in use. Therefore, efforts to overcome this form of resistance tend to focus on this particular protein. Our research is based on the observation that P-gp overexpression makes resistant tumor cells hypersensitive to certain drugs. Our objective was to identify MDR selective compounds, which exploit the function of P-gp. We expanded our earlier efforts to catalogue the National Cancer Institute's DTP drug repository in search of compounds showing increased toxicity in MDR cells. We identified several new candidate MDR-selective compounds in the NCI DTP database. The molecules that were available from DTP were tested on parental and P-gp expressing MDR cell line pairs by viability assays. 10 out of 14 molecules proved to be MDR-selective. In addition, the long-term effect of the most effective compound was further investigated on a resistant colon cancer cell line (HCT-15). Selecting the MDR-positive cell line for two months could lower the amount of P-gp in the plasma membrane. Currently, we are introducing a robotized, high throughput system. We also intend to replace our conventional cytotoxic assay with a cost-effective and more informative fluorescent assay. By using such compounds in tumor therapy the development of resistance could be delayed or abrogated.

Rat model of exercise-induced cardiac hypertrophy – hemodynamic characterization using left ventricular pressure-volume analysis

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Long-term exercise training is associated with characteristic structural and functional changes of the myocardium, termed athlete's heart. Several research groups investigated

exercise training induced left ventricular (LV) hypertrophy in animal models, however only sporadic data exists about detailed hemodynamics. We aimed to provide functional characterization of exercise-induced cardiac hypertrophy in a rat model using the in vivo method of LV pressure-volume (P-V) analysis. After inducing LV hypertrophy by swim training, we assessed LV morphometry by echocardiography and performed LV P-V analysis using a pressure-conductance microcatheter to investigate in vivo cardiac function. Echocardiography showed LV hypertrophy (LV mass index: 2.41±0.09 vs. 2.03±0.08g/kg, $p<0.01$), which was confirmed by heart weight data and histo-morphometry. Invasive hemodynamic measurements showed unaltered heart rate, arterial pressure and LV end-diastolic volume along with decreased LV end-systolic volume, thus increased stroke volume and ejection fraction (73.7±0.8 vs. 64.1±1.5%, $p<0.01$) in trained vs. untrained control rats. The P-V-loop-derived sensitive, load-independent contractility indexes, such as slope of end-systolic P-V relationship or preload recruitable stroke work (77.0±6.8 vs. 54.3±4.8mmHg, $p=0.01$) were found to be significantly increased. The observed improvement of ventriculoarterial coupling (0.37±0.02 vs. 0.65±0.08, $p<0.01$), along with increased LV stroke work and mechanical efficiency reflect improved mechanoenergetics of exercise-induced cardiac hypertrophy. Despite the significant hypertrophy, we observed unaltered LV stiffness (slope of end-diastolic P-V relationship: 0.043±0.007 vs. 0.040±0.006mmHg/µl) and improved LV active relaxation (τ: 10.1±0.6 vs. 11.9±0.2ms, $p<0.01$). According to our knowledge this is the first study, which provides characterization of functional changes and hemodynamic relations in exercise-induced cardiac hypertrophy.

Moderate inhibition of gelatinolytic activity by ilomastat reduces infarct size in both ischemic and reperfusion injury in vivo

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Activation of matrix metalloproteinases (MMPs) is involved in the pathology of acute ischemia/reperfusion injury. Therefore, pharmacological inhibition of MMPs is a promising target for acute cardioprotection.

Aims: In the present study we investigated if the non-selective MMP inhibitor ilomastat given before ischemia or before reperfusion is able to reduce infarct size in vivo.

Methods and results: Infarct-size limiting effect of ilomastat (0.3 - 6.0 µmol/kg) was tested in an in vivo rat model of myocardial infarction induced by 30 min coronary occlusion/120 min reperfusion. Ilomastat at 0.75 and 1.5 µmol/kg decreased infarct size significantly as measured by standard TTC staining, when administered 5 min before the

onset of ischemia as compared to vehicle (DMSO) treated group. When administered 5 min before the onset of reperfusion, ilomastat at 6.0 μmol/kg significantly reduced infarct size. To further assess the cytoprotective effect of ilomastat, primary cardiomyocytes isolated from neonatal rats subjected to 240 min simulated ischemia/120 min of reperfusion were treated with ilomastat (5 nM-5 μM). Ilomastat at 0.5 μM and 5.0 μM significantly increased cell viability when compared to vehicle treated group. To assess the in situ MMP inhibitory effect of the cytoprotective dose of ilomastat (0.5 μM), in separate experiments in situ zymography was performed. Ilomastat showed a moderate (approximately 25%) inhibition of MMP-2 in ischemic/reoxygenated cardiac myocytes.

Significance: This is the first demonstration that MMP-2 inhibition by ilomastat reduces infarct size when administered before the onset of ischemia and before the onset of reperfusion in vivo. The cardioprotective effect of ilomastat involves cytoprotective effect due to a moderate MMP-2 inhibition.

Grafted neuroectodermal stem cells rescue damaged rat retinal ganglion cells otherwise destined to die

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Purpose: Gradual deterioration of vision and progressive retinal ganglion cell loss are the fatal features of glaucoma in human. Our aim was (a) to determine the progressive retinal ganglion cell layer (GCL) loss in an experimental rat glaucoma model and (b) to investigate the neuroprotective effect of intravitreally grafted immortalized murine neuroectodermal stem cells.

Methods: All together 20 (100-150 g, 8-10 w/o) female Sprague-Dawley rats were used. The majority of episcleral veins and the perilimbal venous plexus were thermo-coagulated except a 40-50° segment at the nasal limbus. The right eye was left untreated (intact eyes). Rats that developed corneal or refractive media opacity or severe inflammation were excluded. Transplanted rats (n=5) received neuroectodermal stem cell grafts (500.000 cells/2-3 μm/eye) intravitreally 3 weeks after injury and were allowed to survive for further 6 weeks. Control animals received no graft after the injury and survived for 3, 6 and 9 weeks respectively (n=5 in each group). Intra ocular pressure (IOP) was measured before and after treatment, and then twice a week until the end of the survival period. The operated and intact eyes of all animals were removed at the end of the survival period and the numbers of retinal ganglion cells and optic nerve axons were determined.

The locations of the stem cell derivatives were mapped and their phenotype was immunohistochemically characterized.

Results: IOP significantly elevated after injury but returned to normal levels by 6 weeks after the ocular damage. The number of ganglion cell showed a gradual decrease: 3 weeks: 80.4 % ± 3.1 %; 6 weeks: 75.2 % ± 2 % and 9 weeks: 62.8 % ± 0.6 %. In the transplanted group of animals grafted with stem cells prevent cell death by approximately 50% (surviving ganglion cells: 83.4 %). This finding was confirmed by the number of remaining myelinated axons in the optic nerve: 62.1 % ± 0.8 % in the 9 week control group vs. 80.9 % ± 4.6 % in the transplanted group. (All values are mean ± SEM).

Conclusion: Our results suggest that, grafted neuroectodermal stem cells rescue retinal ganglion cells destined to die due to glaucoma-like injury.

Cooling or heating? Addressing the therapeutic hypotermia from the oxidative stress side

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Introduction: A really efficient neuroprotective therapy is still missing for the acute ischemia reperfusion injury of the brain, but some physical treatment, i.e. cooling the central nerve system can be effective (therapeutic hypothermia). The increase of reactive oxygen species (ROS) production plays an important role in the pathomechanism of these diseases. However, the effect of changing the temperature on the ROS production and elimination isn't cleared yet. In several high prestige journal surprising results were published; that mitochondrial ROS production decreased parallel to rising temperature. In the present study the role of temperature changes were investigated on mitochondrial hydrogen-peroxide (H_2O_2) production and elimination as well as oxygen consumption.

Methods: Mitochondrial H_2O_2 production was measured with Amplex Ultra Red fluorescence, the rate of elimination with H_2O_2 sensitive electrode, respectively. Oxygen consumption of the mitochondria was detected with Clark-type oxygen electrode. All of the measurements were performed on 33 °C, 37 °C and 41 °C.

Results: Using complex I substrate elevation of temperature from 33 °C to 41 °C increased H_2O_2 production by 31,5%. In the presence of complex I inhibitor rotenone an even higher increase of H_2O_2 production (58,8%) was observed. On the other hand the rate of H_2O_2 elimination was also increased parallel to rising temperature, with glutamate-malate substrate it was by 24,2% faster at higher temperature. The oxygen consumption also run parallel with the increasing temperature.

Conclusion: Parallel to rising temperature the rate of oxygen consumption, ROS

production and v elimination was increased. The enhancement of rate of H_2O_2 production was higher than that of elimination, thus the formation-elimination balance with increasing temperature shifted to oxidative stress. Consequently, cooling the central nervous system can contribute to neuroprotection by the decrease of ROS production.

Influence of ultrasonography on performing time and duration of brachial plexus block

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Key words: regional anesthesia, ropivacaine, lidocaine, brachial plexus, ultrasound.

Objective: Evaluation of brachial plexus using regional anesthesia by peripheral neurostimulation or ultrasounds using Ropivacaine 0,5% with Lidocaine 0,5% as anesthetic substances. Study design Prospective, randomized study enrolled 65 adult patients with indication for brachial plexus anesthesia. The anesthetic technique used was either peripheral neurostimulation or ultrasound guided. The processed data included: the quantity of anesthetic, anesthesia installation time, intraanesthetic and postoperatory incidents. Quality and total duration of anesthesia were also recorded.

Results: Brachial plexus anesthesia was performed by peripheral neurostimulation in 40 patients (classic group - CG), and ultrasound guided in 25 patients (ultrasound group - UG). The mean duration of performing the anesthesia was 16, 36 (±7, 21) minutes for UG and 13, 57 (±7, 52) min for CG, p=0, 1453. The mean time of achieving complete motor block was 35 (±14, 9) for the UG and 33 (±9, 41) for CG (p=0, 3293). Total duration of anesthesia was 482.035 (±127, 50) min for CG, and 495,947 (±104, 36) min for UG, p=0, 6954. Ten intra-anesthetic incidents were reported: arterial puncture - 2 in UG (8%) and 8 (20%) in CG (p=0,2939). The quality of anesthesia performed by the two methods was good, insufficient anesthesia being reported in 9 cases (13, 84%), 2 for UG (8%) and 7 for CG (17, 5%) (p=0,4634).

Conclusions: Brachial plexus anesthesia using lidocaine with ropivacaine offers a quality prolonged anesthesia up to 8 hours. Anesthesia performed by ultrasound guided method presents similar performing time, duration, quality and safety degree compared with neurostimulation technique.

Response to Platinum-Based Chemotherapy and Subtype-Specific KRAS Mutations in Advanced Lung Adenocarcinoma

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Purpose: Platinum-based doublet chemotherapy is the most common treatment in advanced lung adenocarcinoma. However, results on the predictive value of KRAS mutation in this setting have been inconsistent. Since the prognostic and predictive role of specific KRAS mutations in pulmonary adenocarcinoma also remain unclear, a mutation subtype-specific analysis was performed.

Patients and Methods: 505 stage III-IV lung adenocarcinoma patients with known amino acid substitution-specific KRAS mutational status and treated with platinum-based chemotherapy were included. The correlations of specific mutations with smoking status, progression-free and overall survival and therapeutic response were analyzed.

Results: Among 338 non-KRAS mutant (67%), 147 codon-12 mutant (29%) and 20 codon13 mutant (4%) patients, there were no mutation-related significant differences in progression-free or overall survival. ECOG status and clinical stage were significant independent prognostic factors in the overall cohort and also within the KRAS mutant subgroup. KRAS mutation showed a significant negative correlation with never-smoker status. Importantly, G12V KRAS mutation was significantly more frequent among never-smokers than the in the other major types of codon-12 mutations (P=0.023). Furthermore, this subgroup tended to have a higher response rate (66% versus 47%, p=0.072). A modest increase was found in the median progression-free survival from 175 to 230 days in the G12V mutant patients.

Conclusions: While KRAS mutation status per se is not a prognostic or predictive biomarker in stage III-IV lung adenocarcinoma, subtype-specific analysis may indeed identify clinically relevant subgroups of patients that ultimately may influence treatment decisions.

Serum chemerin levels and lipid subfractions in obesity

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Chemerin is a recently described adipokine primarily expressed by white adipose tissue. Circulating chemerin levels are significantly higher in obesity, and correlated with various cardiovascular risk factors including lipoprotein levels. To date effect of chemerin on lipoprotein subfractions and its role in the atherosclerotic processes is still unclear. Fifty non-diabetic obese (NDO) patients and 38 age and gender matched lean controls were enrolled. Chemerin level was measured by ELISA. Low-density lipoprotein (LDL) and high-density lipoprotein (HDL) subfractions were detected using non-gradient polyacrylamide gel electrophoresis (Lipoprint). We detected significantly higher serum chemerin level in NDO patients compared to healthy controls (590.1±190.3 ng/ml and 405±127.1 ng/ml, $p<0.001$). Significant positive correlation was between chemerin and LDL-C level ($p<0.001$), and significant negative between chemerin and HDL-C level ($p<0.05$). Significant positive correlations was found between chemerin and the ratio of small dense LDL ($p<0.001$), while negative correlation showed between chemerin and mean LDL size ($p<0.001$). Significant negative correlation was found between serum chemerin and the ratio of large HDL subfraction ($p<0.001$), while there were significant positive correlations between chemerin level and intermediate ($p<0.05$) and small HDL ($p<0.001$) subfraction ratios. Chemerin may have a crucial role in the regulation of lipoprotein metabolism in obese patients without glucose metabolism abnormalities. Early changes in the distribution of lipoprotein subfractions may contribute to enhanced atherogenesis and increased cardiovascular risk.

Comparative investigation of diabetic cardiomyopathy in rat models of type-1 and type-2 diabetes mellitus

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Diabetes mellitus (DM) is associated with characteristic structural, molecular and functional changes of the myocardium, termed diabetic cardiomyopathy. We investigated whether type-1 or type-2 DM lead to different characteristics in cardiac dysfunction, histological and molecular changes.

Methods: Experiments were performed in rat models of type-1 (streptozotocin induced) and type-2 DM (Zucker Diabetic Fatty rats). Detailed characterization of left ventricular (LV) function was performed using a pressure-volume (P-V) conductance catheter system. The slopes of the end-systolic (ESPVR) and end-diastolic pressure-volume relationship (EDPVR) were calculated as load independent indexes of LV contractility and stiffness, respectively. Additionally, TUNEL assay was performed for detection of DNA-strand breaks. Myocardial gene expression analysis was performed by qRT-PCR, expression of proteins was investigated by western blot and immunohistochemistry.

Results: When compared to control, type-1 DM was associated with decreased LV systolic pressure, maximal dP/dt, Ees (1.04±0.07 vs 2.23±0.20 mmHg/µl) and cardiac and stroke work indexes. In type-2 DM, systolic indexes were altered only to a lower extent and the increase of LV stiffness was more pronounced (slope of EDPVR: 0.058±0.004 vs 0.019±0.004 mmHg/µl). Histological examination showed hypertrophy and degeneration of cardiac tissue. DNA-damage, nitro-oxidative stress, overexpression of c-fos and c-jun and downregulation of eNOS were more pronounced in type-1 DM. On the other hand TGF-β1 and ANF mRNA-levels were markedly upregulated in type-2 DM.

Conclusions: Diabetes is characterized by decreased systolic performance and delayed active relaxation (mainly in type-1 DM), accompanied by increased diastolic stiffness (mainly in type-2 DM). Correspondingly a different pattern and severity of myocardial structural and molecular changes could be documented between the two models. (Supported by the grant OTKA PD100245)

Sampling the binding of Berberine to human telomeric G-quadruplex DNA through Funnel-Metadynamics simulations

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The isoquinoline alkaloid Berberine inhibit telomerase activity[1] binding G-quadruplex DNA by mean of its aromatic moiety that is able to form stacking interactions with G-quartets (pdbcode 3r6r).[2] However, the details about the functional mechanism and the binding behaviour of this molecule with G-quadruplex DNA are not fully understood. This information can be obtained using enhanced sampling techniques such as metadynamics (MetaD).[3] MetaD plays a leading role in studying ligand/protein binding

mechanism[4] and, moreover, has also been successfully applied to study G-quadruplex folding.[5] The sampling is enhanced by the addition of a bias potential that acts on a selected number of degrees of freedom, known as collective variables. The very recently developed formalism funnel-metaD [4] has been applied on the G-quadruplex/Berberine complex to get further insight on the binding process. We reconstructed the binding free-energy surface with an accurate estimation of the ligand/protein absolute binding free energy. Our results have revealed an alternative binding mode of Berberine to G-quadruplex and elucidated the crucial role of waters during ligand binding. Our study represents the first application on DNA of this newly developed method, providing important information for designing new G-quadruplex antitumor ligands.

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Multifractal analysis of near-infrared spectroscopy (NIRS) signals recorded from the human brain cortex

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$1/f$ noise has been found ubiquitous in natural processes. This fractal pattern can be modeled in the frequency domain as $|A|^2$ $1/f$ where β (spectral index) captures the scale-free behavior of power amplitudes (A) within the scaling range (SR) of frequencies (f). Using multifractal formalism, the global distribution of the fractal measure can be revealed in a singularity spectrum, $D(h)$. Previously, we reported that NIRS-signals did spontaneously fluctuate in the human brain cortex as monofractal processes. Our aim was to investigate if these hemodynamic signals have multifractal properties.

NIRS-signals (sampled at 2 Hz, for 8192 s) were obtained from the prefrontal cortex of healthy volunteers (4 groups with n=5 in each: young male - M1, young female - F1, aged male - M2, aged female - F2). The analysis was carried out in time domain with the

differential Multifractal Detrending Moving Average method. The end-point of multifractal analysis, P_c, was calculated from characteristic measures of $D(h)$.

Fractal measures pertinent to SR were obtained (β F1:1.10±0.25, M1:1.16±0.13, F2:1.63±0.35*, M2:1.01±0.35*; P_c F1:0.03±0.03, M1:0.03±0.02, F2:0.15±0.15*, M2:0.02±0.01*; *:p<0.05), and significant differences (two-way ANOVA, Bonferroni post-hoc test) were found between F2 and M2 groups both in β and P_c.

To conclude, we demonstrate that (i) multifractal temporal structuring is present in hemodynamic fluctuations recorded from the human brain cortex, (ii) in the post-menopause age groups, gender has an impact on mono- and multifractality, which can be explained by vascular (age-related stiffening of cerebral vasculature) or neural factors (declining neural activity).

Behavioral treatment of obesity

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Introduction: Behavior therapy is one of the evidence based methods in the treatment of obesity. Standard behaviour therapy for obesity was extended with self-help. The aim of our study was to determine the feasibility and the results of such a setting.

Methods: The 24-week weight loss program involved 39 participants (10 men and 29 women) between 21- 65 years of age (M= 39,9, SD=11,25) Patients's mean BMI was 37,4 (SD=8,06). The overweight participants were mentally sine morbo. Exclusion criteria were psychiatric disease and chronic physical illness. The effectiveness of this therapy was assessed by measuring the body weight of participants, and by participants filling in questionnaires before the first session and after the 8th, 16th and 24th sessions. We used the following measures: Three-Factor Eating Questionnaire, Rosenberg Self-esteem Questionnaire.

Results: Respondents reported average weight loss to be around 9.9 kg (SD=5,87, range: 2,0-27,0 kg), which corresponds to 8,8% (SD=4,31%, range: 2,1-19,1%) of baseline weight. 90% of participants achieved the normative of a 5% minimum weight loss during treatment. 28% attained 10% weight loss. Significant relationships (p<0,001) and large effect sizes were found regarding changes of eating behaviour (such as uncontrolled eating, emotional eating, cognitive restraint) (Cohen's d =1,80-3,04). Significant relationship was found between the increase of cognitive restraint (r=0,51;

p=0,002) and the decline of emotional eating (r=0,38; p=0,021) in respect to the rate of weight loss during treatment. A tendency was found between the decline of uncontrolled eating and the rate of weight loss (r=0,31; p=0,058). Weight loss was associated with improvement of global self-esteem (β=0,43; p=0,009), which remained significant even when controlling for background variables (β=0,34; p=0,033).

Conclusion: Our results suggest that standard behavioral therapy extended with self-help elements can be a cost - efficient method to help obese patients reduce weight. Treatment has significant benefits in changing maladaptive eating behaviours into adaptive ones, and these changes resulted in bigger weight loss during therapy. Intentional weight loss has a favourable effect on mental health, which is shown by the relationship between weight loss and global self-esteem.

CT radiation exposure: looking back at the last decade

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Background: The introduction of Computed Tomography (CT) has transformed diagnostic radiology. Since the 1970's its use has augmented rapidly; globally there was a four times increase of the number of scans per patient in the last decade.

Material and Method: We considered the CT exams performed in the Radiology Laboratory of Tirgu Mures Clinical Emergency Hospital from 2003 to 2012 with regards of the referring physicians and demographic patient data. MS Office and GraphPad Prism 5 were used for data analysis of the samples.

Results: While the number of required studies increased constantly from 5678 to 12947 there are large discrepancies among the referring specialties. When considering the total patient dose there is a significantly ascending linear trend especially for the emergency room patients (p=0.002).

Conclusions: Patient addressability had increased in the last 10 years providing a significant increase in population irradiation. A stronger enforcement of the ALARA principles correlated with a good communication with the ordering physicians is required.
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Assessment of fitness and lifestyle habits in medical students

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Background: Although regular physical activity and healthy eating habits are part of the general prevention recommendations, the great majority of people nowadays tend to apply them only after disease occurs. Attention should be focused on healthy lifestyle since childhood throughout adult life and also in the elderly.

Objective: The aim of the study was assessing lifestyle habits of the medical students and the relations with their fitness.

Material and Methods: Type of study – cross-sectional. Target population – the students of UMF Tirgu Mures. Covenience sample – 57 volunteers who filled in a questionnaire and underwent the Harvard step test, with consequent calculation of the fitness score (FS). The statistical analysis was performed using M.O. Excel and GraphPad.

Results: Healthy eating habits – at least 3 meals/day and regular breakfast are associated with significant better FS (p=0.01 and p=0.04 respectively). Only 5 (8.9%) subjects claimed to consume the recommended number of fruit and vegetables servings/day. Smokers have significantly lower FS (68.18 vs. 76.1, p=0.02). Physical activity intensity or frequency didn't have a significant influence on the FS for this sample. 16/57 subjects had good FS and the same percentage are already overweight or with abdominal obesity

Conclusions: More attention and time should be paid to physical activity and healthy eating habits from college years. A randomized trial would be useful for assessing the need for more mandatory sport classes and prevention medicine. Acknowledgement: This paper is partly supported by the Sectorial Operational Program Human Resources Development (SOP HRD), financed from the European Social Fund and by the Romanian Government under the contract number POSDRU 80641. Project partly supported through an Internal Research Grant of University of Medicine and Pharmacy Tirgu Mures.

Disclosure: Partial results were presented at The 17th International Scientific Congress for Students, Young Physicians and Pharmacists Marisiensis, 17-21 April, Tirgu Mures.

Keywords: Harvard Step Test, Fitness Score, Anthropometric Parameters

Genetics of suicidal behavior: role of the microRNA system

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Suicide is the most devastating outcome of the psychiatric disorders, its possible risk factors have been studied intensively. Recent studies reported an association between the executive functions and suicidal behavior that has been replicated repeatedly and was independent from the psychiatric diagnoses. The genetic architecture of the executive functions is still unclear, however, the microRNA (miRNA) system has been implicated by multiple studies. The miRNAs play a vital role in neurodevelopment and synaptic plasticity, influencing the executive functions. The objective of our study was to examine the gene-based associations between the suicidal behavior and miRNA genes differentially expressed in the dorsolateral frontal circuitry among subjects with major depressive (MDD) and bipolar disorder (BD). Samples from NCBI GAIN BD (n=999) and MDD (n=1753) case-control datasets were analyzed. Suicidal behavior was defined by having severe suicidal ideation, or lifetime suicide attempt, control subjects were non-suicidal BD or MDD cases. Genes were selected by using the EBI Gene Expression Atlas, 53 of the 91 miRNA genes were sufficiently covered. Gene-based association tests were assessed with VEGAS and were validated with KGG. Datasets were combined in a random effect meta-analysis and population stratification was corrected with the genomic inflation factors. Multiple comparisons was corrected by using permutaion with 1000,000 cycle. Nominal significant p-values were detected for DICER1 (p=0.0042) and MIR765 (p=0,0253) genes, and DICER1 remained significant after the correction for multiplicity (p=0,048). The product of the DICER1 gene is a rate limiting factor in the biosynthesis of the miRNAs and suggested to have a significant effect on the executive functions. The statistically significant association between the DICER1 gene and suicidal behavior found in this study in both the MDD and bipolar sample corroborates the link between executive functions and suicide cutting across DSM-IV diagnoses and may contribute the better understanding of the etiology.

New potential folds of G-quadruplex DNA investigated through MonteCarlo simulations

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Today is widely recognized the polymorphic nature of nucleic acids, which allows such structures to adopt different conformations from the well known forms double-stranded, such as that of G-quadruplex.[1] It is formed from G-rich sequences, such those of telomeres and promoters of oncogenes, and it is built around tetrads of hydrogen-bonded guanine bases.[2] Recently, for the first time, such structure has been explicitly proved within the nuclei of cells of tumors, therefore, now it is considered as an attractive anticancer drug target.[3] Drugs able to bind to stabilize G-quadruplexes can be employed to suppress the elongation of telomers, the gene transcription and translation of oncogenes.[4] However, the *drug design* is made difficult by the polymorphism of this structure.[5] Indeed, for the unimolecolar G-quadruplex, based on human telomeric sequence $d(TTAGGG)_4$, we have to consider four different folds experimentally-determined: parallel (pdbcode 1KF1), hybrid 1 (pdbcode 2HY9 and 2JSM), hybrid 2 (pdbcode 2JPZ and 2JSL) and antiparallel (pdbcode 143D). Other folds not experimentally-determined, which effect biological, chemical and physical behavior of this molecule can occur. Therefore, a deeper conformational study of the target is crucial to improve the information about this target. With this aim an intensive Monte Carlo approach is currently adopted in our lab.

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New Aspects of the Clinical Course in Otogenic Lateral Sinus Thrombosis: 15 Year Experience at a Tertiary Level Pediatric Hospital in Hungary

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Objectives: In Hungary the majority of children are vaccinated against Haemophilus influenzae type b and Streptococcus pneumoniae. Excess to antibiotics is good. Despite these facts lateral sinus thrombosis as a serious intracranial complication of otitis media occurs. Our goal was to describe some novel facts in the clinical picture of children treated with otogenic lateral sinus thrombosis in a tertiary level pediatric hospital from 1998-2013.

Patient and Methods: The medical charts of 10 patients were reviewed.

Results: All patients experienced acute otitis media before admission. Half of them were treated with antibiotics weeks before admission. By them the neurological signs dominated the clinical picture at admission. Those, who were not treated with antibiotics, experienced the thrombosis in the acute stage of the illness with otogenic symptoms, sepsis, and without focal neurological signs. At admission by all patients the fundoscopic examination revealed papilledema, but by none of them could visual impairment be detected. After mastoidectomy, by 7/10 children the eye back ground worsened, new haemorrhages appeared and two children complained for visual loss. At one year follow up only by one patient was a permanent visual loss diagnosed on one eye.

Conclusions: Lateral sinus thrombosis as a serious intracranial complication of otitis media can even after vaccination against Haemophilus influenzae type b and Streptococcus pneumoniae with good excess to antibiotics occur. The signs of raised intracranial pressure worsen after mastoidectomy. So the visus and eye background of the patients must be checked regularly postoperatively, and intervention must be applied promptly, if it worsens.

Reduced neural baroreflex-sensitivity is related to enhanced endothelial function in patients with end-stage liver disease

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Introduction: Reduced cardiovagal baroreflex-sensitivity (BRS) was found to be an independent predictor of mortality in end-stage liver disease, but the underlying mechanism of impaired baroreflex function is not known. BRS consists of two components: the mechanical component (mBRS) is determined by the distensibility of the baroreceptor vessel walls and the neural component (nBRS) reflects the sensitivity of the neural signal processing. In animal models nitric oxide (NO) modulates neural responsiveness of the baroreflex arch elements in a dose-dependent manner. Increased endothelial NO production in end-stage liver failure might decrease nBRS. Therefore, we investigated if (i) the reduction of mBRS and/or nBRS is responsible for the impaired baroreflex function (ii) enhanced endothelial function influences nBRS.

Methods: We enrolled 20 patients with end-stage liver disease and 17 age- and gender-matched controls. BRS was determined by ECG and beat-to-beat blood pressure recordings. mBRS was characterized by the distensibility coefficient of the common carotid artery (DC). nBRS was estimated by the ratio of BRS and DC. The endothelial function was quantified by flow mediated dilation of the brachial artery (FMD).

Results: BRS was markedly reduced in patients compared with controls (8.5±2.7* vs. 13.5±9.3 ms/Hgmm). The mBRS was not different in the two groups (2.5±0.8 vs. 2.4±0.8 10-3/Hgmm), while nBRS showed significant reduction in patients (3.6±1.5* vs. 5.5±3.0 ms/10-3). FMD was higher in patients with end-stage liver disease (9.9±4.0† vs. 5.9±1.3 %). FMD and nBRS was inversely related in patients (r=-0.51*), but directly related in controls (r=0.62*). (mean±SD; *:p<0.05; †:p<0.01)

Conclusion: Reduced BRS in end-stage liver disease can be explained by the deterioration of nBRS, and mBRS appears to be preserved. Endothelial NO may enhance BRS in healthy subjects, however, central, endothelial overproduction of NO possibly contributes to the reduction of nBRS in patients with end-stage liver disease.

Seizure prediction in Epilepsy by CNN

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Epilepsy is the most common chronical disorder of the neurological system. 1% of the world's population suffers from recurring epileptic seizures, and many of them cannot be treated with medication. Thus, the aim of investigations by various authors from multiple fields of research is the realization of a miniaturized, implantable seizure warning device. This would not only improve the quality of life for patients whose seizures cannot be controlled by medication, but could also open up new possibilities of treating seizures while minimizing side effects of drugs. Cellular Neural Networks (CNN) were introduced by Chua and Yang in 1988 and have been an active topic of research within the seizure prediction research community. Due to their inherently massive parallel processing power they form an attractive basis for the realization of a miniaturized seizure warning device. Here, we propose an auto/cross correlation based approach allowing a calculation time efficient determination of CNN coupling weights and its application to the data of several patients. An analysis with special respect to the influence of the polynomial order of coupling weight functions on the prediction error will be presented. Additionally, first results of an out-of-sample study investigating the performance of signal feature extraction methods based on the detection of interdependencies between different brain regions will be given.

Investigation of the direct effect of complement factor B on platelet-aggregation

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Introduction: Thrombotic thrombocytopenic purpura (TTP) is a thrombotic microangiopathy. Characteristic for this disease is the consumption of platelets due to their activation in small vessels. Even though severely decreased activity of the enzime ADAMTS13 is observed in most cases, there is no clear explanation, why the lack of enzime-activity alone is not sufficient for the development of the disease. Beyond the ADAMTS13-deficiency, other triggering events are needed for the manifestation of the disease. Last year we presented the results on the connection between complement-activation, a possible triggering factor, and platelet-activation during the acute phase of TTP. The extent of the latter was indicated by a value called „platelet factor 4 excess". In

our experiment, this index positively correlated with the levels of complement factor B, factor H, factor I and the soluble terminal complex. Complement factor B is a serine protease, therefore we hypothesized that it may directly activate platelets by cleaving their PAR receptors. According to this hypothesis, the aim of our present experiment was to investigate the direct effect of complement factor B on platelet-aggregation.

Methods: Our measurements were carried out using platelet suspensions isolated from blood samples of healthy subjects. The in vitro aggregation of platelets was provoked by PAR agonist TRAP-6. The extent of platelet aggregation was determined by measuring light-transmission. In 4 of the 16 cases, complement factor B was added to the samples directly before starting the aggregation, in another 4 cases, the samples were incubated with factor B for 15 minutes prior to the measurement. The concentration of factor B was 111nM in both cases, the concentration of TRAP-6 was 8μM in all of the samples.

Results: The rate of platelet-aggregation increased sharply after TRAP-6 application, until it reached a maximum of 65-80%. Applying factor B did not alter the rate of platelet aggregation, neither when added directly before, nor when added 15 minutes prior to the measurement.

Conclusions: According to our experiment, complement factor B does not directly affect the activation of platelets, so there may be other mechanisms explaining the connections between complement-activation and platelet-activation during the acute phase of TTP.

Genetic determinants and herpes viruses in the background of periodontitis in the Hungarian population

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Objectives: Periodontitis is a complex multifactorial disease: genetic factors, pathogenic bacteria, environmental factors and herpes viral infections have been shown to be involved in its etiology. We investigated 16 single nucleotide polymorphisms (IL-1α-889, IL-1β+3954, IL-1β-511, IL-10-1082, TNFα-308, TLR4-299, TLR4-399, VDR-1056, TNFα-1031, IL-10-597, IL-6-1363, CD14-260, COX2-8474, ASPORIN-9659, MMP8-799, ANRIL) and 5 herpes viruses (HSV1-2, VZV, CMV, EBV) in the Hungarian population.

Methods: DNA was isolated from buccal scrapings from 355 patients. They were classified according to clinical parameters into healthy, gingivitis, chronic and aggressive periodontitis groups. SNPs were identified by Genotyping Realtime PCR, viruses were detected by nested PCR. Group-wise differences were calculated by logistic regression, Chi2 probe, and were further analysed by Bayesian multilevel statistical analysis.

Results: We observed significant differences in allele frequencies of the IL-1β-511, IL-6-1363, TNFα-1031 and CD14 SNPs and in the genotype distribution of polymorphisms within the IL-1α-889, IL-1β-511, IL-6-1363, TNFα-1031, CD14, Asporin and Anril genes. VZV showed higher incidence in aggressive periodontitis compared to controls. By the Bayesian method we created a possible dependency model in the background of periodontitis in the Hungarian population.

Conclusions: Among the SNPs investigated CD14-260 was the only central factor in the development of periodontitis in the Hungarian population, through which TNFα-1031 and the two TLR4 SNPs acted, while herpes simplex viruses have an independent effect. The use of our model may result in novel diagnostic and therapeutic approaches in periodontology.

Possible anti-tumorigenic usage of angiostatin in oncotherapy

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Introduction: Angiostatin, a cleavage product of plasminogen kringle domains, is an endogenous angiogenesis inhibitor which can decrease tumorous growth. However, angiostatin is instable so therapeutic usage is complicated. Our goal was to design an angiostatin-protease chimera which produces angiostatin in situ and in vivo after activation.

Methods: PCR amplified protease, angiostatin cDNA and elements needed for expression in bacterial and eukaryotic cells were ligated into an adequate vector in the same reading frame. The plasmid was used for transfecting different cell lines to archieve in vitro and in vivo production. Aorta ring assay served for monitoring endothelial proliferation in presence of transfected cells. BALB/c mice were injected with transfected and wild type 4T1 breast cancer cells to quantify tumor growth in time.

Results: The chimera was purified from E. coli and its autocatalysis was observed using SDS-PAGE: the full chimera (80 kDa) appeared if isolation happened in presence of protease inhibitor. Endothelial proliferation in hanging drop culture stopped when chimera expressing cells grew at the bottom. The aorta ring assay became negative on transfected cell lines. In vivo tumor progression was tested using 20 independent clone lines. Mice

injected with transfected 4T1 cells only started growing cancerous tissue 10 days after infection and their tumor progression proceeded with slower rate compared to controll animals. Metastases occuring in these mice showed smaller diameters as well.

Conclusion: Our chimera cleaves active angiostatin and develop its anti-tumorigenic effect as demonstrated both in vitro and in vivo experiments. Patent application is in process.

Decreased paraoxonase 1 (PON1) lactonase activity in hemodialyzed and renal transplanted patients

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Background: Human paraoxonase-1 (PON1) has also been described as a lactonase. Decreased PON1 lactonase activity was found to be a predictor of cardiovascular disease. Homocysteine thiolactonase (HTLase) activity may prevent proteins from homocysteinylation and is thought to be a protective factor against the progression of atherosclerosis. Previous studies have demonstrated decreased PON1 paraoxonase activity in hemodialyzed (HD) and renal transplant (TRX) patients; however lactonase activity has not been investigated. We aimed to determine the paraoxonase and lactonase activities, and to clarify the relationship between lactonase activity and a set of cardiovascular risk factors, such as homocysteine, cystatin C and asymmetric dimethylarginine (ADMA) levels in HD and TRX patients and in healthy controls.

Methods: 108 HD and 78 TRX patients, and 63 healthy controls were involved in the study. Paraoxonase and lactonase activities (paraoxon and gamma-thiobutyrolactone as substrates) were measured spectrophotometrically. ADMA level was determined with sandwich ELISA.

Results: Both HD and TRX patients had significantly lower lactonase activities compared to the control group (<0.05). Significantly lower paraoxonase activities were found in HD patients compared to the TRX group ($p<0.05$). Significant negative correlation was found between lactonase activity and ADMA level in the whole study population ($p<0.001$), while paraoxonase and lactonase activities showed significant positive correlation ($p<0.001$). Multiple regression analysis identified paraoxonase activity and homocysteine level as independent predictors of lactonase activity.

Conclusions: Lactonase activity is a potential new predictor of cardiovascular risk in renal failure. Measurement of lactonase activity is recommended in future studies on HD and TRX patiens.

Clinicopathological features of head and neck cancers

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Introduction: Prognosis of head and neck squamous cell carcinomas (HNSCCs) is considerably poor (5-year overall survival: 50%). In our study, we compared the biomarker-expressions of HNSCCs arising from different anatomical localizations and evaluated their correlations with the clinical parameters and the survivals.

Methods: 226 patients having HNSCCs were enrolled into our study. The tumors' distribution by localisation: 12 oral cavity, 69 oropharyngeal, 43 hypopharyngeal, 43 glottic, 42 supraglottic und 17 transglottic. We prepared Tissue Microarrays (TMA) from the patients' tissue samples and used immunohistochemical staining for detecting the biomarkers (Ki67, p53, EGFR, p16ink4, Collagen XVII). The histological evaluation was performed on computers using the Panoramic Viewer software. Expressions were considered positive when more than 25% of tumor cells were stained. We performed Chi-square tests and Kaplan-Meier survival analysis.

Results: The survival rate of patients with p16-negative cancer was significantly lower than that of patiens with p16-positive disease. The Ki67-positivity was associated with worse prognosis and a higher rate of lymph node metastasis. Factors such as T and N stage, diameter of the tumor and the histological grade significantly affected the prognosis.

Conclusions: According to our study we can declare that the supplementation of the conventional TNM-system with biomarker-profile could lead to a more precise prognostic score. This can help us to choose the most suitable therapy for our patients.

Spine examination of primary school children with Zebris ultrasound-based motion analysing system

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Introduction: Since 2005 different motion analysing researches have taken place in the biomechanical laboratory of MÁV Hospital in Szolnok. A major area of our research is to determine the spinal shapes of primary school children with ultrasound-based motion analysing system.

Aims: The goal of our research is to survey the spinal conditions of children reckoned to be healthy with physical examination and to determine the shape of their spines with ultrasound-based motion analysing system. Another aim is to determine – through a large number of examinations – reference values (kyphosis, lordosis, inclinations) which specify the shapes of spines for different age groups.

Method: Subjects were 210 – from six to twelve years old – children, who were examined every half year for four years. First the subjects were measured in standing position and the spatial coordinates of the processus spinosuses were located with WINSPINE software specially designed for Zebris CM-HS motion analysing system. Than from these data we calculated the changes of thoracal kyphosis, lumbal lordosis and inclinations (total trunk inclination, lateral inclination) both in frontal and in sagittal planes.

Results: After the physical examinations we divided the children into four groups (healthy children, children with bad postures, children with flat backs, and children with scoliosis). In the research we were able to define reference values for every age group. Through the examinations it revealed that even at healthy-reckoned children posture disorders could be diagnosed which cause no pain or discomfort during childhood, but later could worsen and could be the sources of several rheumatologic and orthopaedic diseases. From large number of examinations we succeeded to establish a new reference set, which is slightly different from the values used so far.

Measurement of serum total cortisol using high performance liquid chromatography (HPLC) coupled esi-tof mass spectrometry (MS)

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Cortisol is a glucocorticoid hormone with low molecular weight (362 Dalton) synthesized from cholesterol in the adrenal gland. The release is regulated by the hypothalamic-pituitary-adrenal (HPA) axis. Approximately 95% of the circulating amount is bound to proteins (CBG, albumin), but only the remaining free fraction is biologically active.

Serum cortisol level is routinely analysed in laboratory medicine, though the widespread immunoassays (RIA, ECLIA) have the disadvantage of cross-reactivity with some commonly used steroid drugs, which can be eliminated by MS. On the other hand in routine diagnostics we only have the opportunity to measure total cortisol concentration, but the identification of free cortisol provides more informative results. MS has become a method

of increasing importance for cortisol estimation because after suitable sample preparation it provides the measurement of free fraction as well.

Our aim was to develop a mass spectrometric method to analyse serum total, serum free, and salivary cortisol based on accurate mass identification, which can be reliably used in diagnostics and in therapy follow-up.

The analysis was carried out on a Bruker micrOTOF mass spectrometer using deuterated (9,12,12-D3) cortisol as internal standard. Sample preparation involved protein precipitation, serum ultrafiltration, and solid phase extraction.

The limit of detection (LOD) for total cortisol measurements was 9 nmol/L and the limit of quantification (LOQ) was 15 nmol/L. The calibration curve was linear from 25 fmol to 104,6 pmol (on column). Average intra-assay variation was 3.1%, while the inter-assay variation was 6.3%. Results were compared with the data of the Roche Modular Analytics E 170 ECLIA assay. The comparison resulted in eligible correlation (R2=0.96, slope=0.9725, CI 0.971-0.991 at 95%) in every (low, medium, high) concentration range.

We can conclude that MS coupled with HPLC has higher specificity compared to immunoassays as identification is based on compound mass, instead of structural characteristics. We did not observe any interference with the therapeutically used steroid drugs, which is a common limitation of the immunoassays. Our method is capable of specific cortisol quantification in different matrices based on accurate mass identification.

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Passive-transfer-trauma model for the Complex Regional Pain Syndrome (CRPS) in mice

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Complex Regional Pain Syndrome (CRPS) is a chronic persistent pain condition with redness, warmth and swelling of the affected area occuring after a minor limb injury mostly in women. Since its mechanism is unclear and there is still no cure, an appropriate

animal model for experimental investigations is needed to analyse the pathophysiological processes. Important roles of immune responses, sensory/sympathetic nervous systems and complex neuro-immune interactions are suggested to be involved in its mechanisms.

Passive transfer-trauma model of CRPS was set up in female C57Bl/6 mice with a small plantar skin-muscle incision followed by repeated treatment with purified serum-immunoglobulin G (IgG) of CRPS patients and healthy volunteers. Mechanical pain sensitivity, paw volume, motor coordination and paw temperature were measured during 8 days. Sensory nerve-derived neuropeptides and inflammatory cytokines were determined from the paw homogenates.

CRPS-IgG injection significantly increased incision-induced paw oedema and touch sensitivity (hyperalgesia) compared to the healthy-IgG-treated group, but did not influence motor coordination and paw temperature. CRPS-IgG remarkably and selectively elevated the concentration of the sensory neuropeptide substance P (SP) in the paw, but inflammatory cytokines produced by immune cells remained unchanged.

These data provided evidence that IgG plays a crucial role in CRPS-related edema and pain, in which predominantly sensory neural mechanisms and SP are likely to be involved. Since the characteristic clinical signs of CRPS could be successfully transfered to mice, this model is suitable for identification of mechanisms, key mediators and targets for novel therapy.

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A systematic analysis of the complement pathways in the patients with neuromyelitis optica indicates alteration but no activation during remission

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Neuromyelitis optica (NMO) is an autoimmune disease of the central nervous system characterized by inflammation of the optic nerve and spinal cord. The disease is mediated by autoantibodies against aquaporin 4, the main water channel of the central nervous system. Local IgG deposition and complement activation within the CNS is pathological hallmark of the disease. Autoantibodies are present in the circulation, however data on the complement system are scarce, detailed analysis of the complement system hasn't been done. In the study 25 AQP4-seropositive patients in remission phase (40 (30-69) age, 22 female) and 113 (35 (30-43) age, 64 female) healthy volunteers were involved.

All patienst were treated by azathioprine. Activities of the complement pathways were measured in sera by the hemolytic titration (classical pathway) and Wi-ELISA (alternative and lectin pathways). Serum levels of C1q were determined by ELISA system. Serum C3 levels were measured by turbidimetry, C4, C5, factor B, factor I, properdin and C1-INH concentrations were determined by radial immunodiffusion. The plasma levels of complement activation products C1rC1sC1-INH, C3a, C3bBbP and SC5b-9 were determined by ELISA system. Activities of both the classical and lectin pathway were significantly higher in patients ($p=0.0122$ and $p=0.0078$) than in the controls, however the activities of the alternative pathway didn't show significant elevation. Levels of C3, factor I, factor B and properdin were significantly lower ($p<0.0001$, $p=0.0007$, $p=0.0397$ and $p= 0.0028$) in NMO patients than in the controls. By contrast neither C1q, C1-INH, C4 or C5 levels differed between the two groups. We didn't find significant elevation of C1rC1sC1-inh levels in EDTA-plasma, however levels of C3a, C3bBbP and SC5b-9 were significantly lower ($p=0.0006$, $p<0.0001$ and $p=0.0128$) in the samples of patients compared to controls. Although the specific autoantibody is present in the circulation of the patients our results do not indicate any substantial complement activation in adequately controlled patients; nevertheless, the data indicate differences between patients with NMO and healthy controls, suggesting that the complement system is abnormally affected even during remission and treatment. Systemic complement studies might be of diagnostic value in NMO.

POSTER PRESENTATIONS – PHARMACEUTICS

2-(2-cycloalkylidenehydrazin-1-yl)-4-aryl-1,3- thiazoles: a promising class of antifungal agents

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C. albicans is an opportunistic pathogen that can be found in the 50% of the healthy population. However, when the immune system is compromised or the balance of the endogenous microflora is altered, the microorganisms often become pathogenic. While in immuno-competent patients causes superficial and moderate infections, in immuno-compromised individuals it can also cause systemic infections by spreading in the blood stream and invading the internal organs [1]. For many fungal infections, polyenes, such as amphotericin B, represent the standard therapy. Polyenes bind to membrane sterols, leading to membrane permeability, leakage and cell death. However, the clinical use of amphotericin B is limited by a high frequency of renal toxicity and several adverse effects. Azoles, such as fluconazole, which act on ergosterol biosynthesis, offer several advantages over amphotericin B in terms of decreased toxicity after oral or intravenous (iv) administration and are often employed in the treatment of fungal infections sustained by Candida spp. However, failure of fluconazole has been reported and acquired or intrinsic resistance to fluconazole has been described. [2] New semisynthetic echinocandins, such as caspofungin and micafungin, are fungicidal water-soluble molecules that inhibit synthesis of 1,3-b-D-glucan, a main structural component of the fungal cell wall.[3,4] However, echinocandins are expensive and often not affordable, in particular in low income countries. Because of the importance of fungal infections, in particular in immuno-compromised individuals, the limitations of currently available antifungal agents regarding their toxicities, and the increasing prevalence of pathogen resistance, new fungicidal agents are needed. In this poster presentation we wish to report on the results of our research on the design, synthesis and biological activity, towards fluconazole resistant Candida spp., of a series of 2-(2-cycloalkylideehydrazin-1-yl)-4-aryl-1,3-thiazoles.

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In vitro permeability studies for nasal drug delivery using „side-bi-side" horizontal cell model

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Intranasal administration is a potential way to deliver drugs into the systemic circulation as an alternative route for some therapeutic agents [1]. The development of nasal formulations open up new areas of indication and many delivery problems can be solved. The large surface area of the nasal mucosa affords a rapid onset of therapeutic effect, potential for direct-to-central nervous system delivery, no first-pass metabolism, and non-invasiveness does not require sterile preparation; all of which maximize patient comfort and compliance [2]. The drug diffusion from nasal formulations is examined with in vitro and ex vivo experiments. The aims of our work were to optimize the method of measurement (setting device, sample preparation and sampling), and investigate and compare the diffusion of different pharmaceutical formulations for nasal delivery (gel, dry powder and viscous liquid form). „Side-bi-side" diffusion system was applied for in vitro examination of formulations. Impregnated, artificial membrane was used between donor and acceptor compartments. The model drug was meloxicam. It was concluded that the „side-bi-side" horizontal diffusion system is suitable for permeability studies of formulations for nasal delivery. The small volume of compartments (3 ml) well simulates the nasal conditions. Primarily the investigation of nasal sprays and powders is possible with the method discussed above. The „side-bi-side" system is suited to ex vivo permeability studies as well, to investigate the diffusion through mucous membranes prepared from animals.

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Formulation and characterisation of voriconazole-loaded intravenous nanoparticles

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In the past few years human serum albumin (HSA) is instrumental in drug development for new carrier system of active pharmaceuticals ingredient (API). Intravenous liquid dosage form development of drugs or drug candidates that show high plasma protein binding and poor water solubility can be solved by using human serum albumin. Contrarily to conventional excipients it is biocompatible and well tolerated by the human organism without any serious side-effects, such as toxicity or allergic reaction. Day after day the number of the systemic fungal infections is ever increasing and thus their treatment has undergone significant changes in the last years, mostly owing to the new antifungal agents. Voriconazole, a triazole type antifungal agent used as the first line of treatment of invasive aspergillosis, was selected as the model active pharmaceutical ingredient. As voriconazole exhibits poor water solubility and high binding affinity to HSA it is a very promising candidate for the development. Aim of our study was to prepare an injectable liquid formulation using nanoparticle albumin-bound technology (NAB-technology), a technology based on the formation of nanoparticles comprising HSA and selected API. Voriconazole-loaded nanoparticles were developed by high pressure homogenizer (Emulsiflex B15, Avestin, Canada). Process parameter optimization led to the elaboration of the optimal settings: six homogenizing cyles at 1800 bar pressure. The physicochemical features of formulated nanoparticles was investigated by Zetasizer Nano S (Malvern, United Kingdom) such as particle size distribution and polydispersity index. The average size in the optimized product was 48.65 nm which is allowed to administrate parenteral. Furthermore, NAB-voriconazole particles resulted in the solubility enhancement of the drug by at least twofolds. In vitro dissolution modell is suitable for predicting the in vivo release behavior of the voriconazole nanoparticles. In our study half the amount of the totally encapsulated voriconazole was liberated in less than an hour. The concentration of the API was determined by HPLC-UV following the sample preparation, which was developed specifically for the samples and included precipitation and centrifugation.

Simultaneous determination of antiepileptics in various biological fluids using LC-MS/MS method

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Aims: The main purpose of the study was the development of a specific and precise method for the quantification of different third generation antiepileptic drugs to evaluate their specific pharmacokinetic properties – tissue distribution, blood-brain-barrier passing rate – during concomitent use. A rapid and sensitive liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) method was developed for rufinamide and lacosamide in various plasma samples and validated according to the current bioanalytical guidelines.

Materials and methods: The separation was conducted on a reversed phase column (Zorbax SB-C18 100 x 3 mm, 3.5 µm), the mobile phase consisting of methanol and 0.1% formic acid in water (50:50 v/v) at 40 °C with a flow rate of 0.3 mL/min. The detection of the analytes was performed with an Agilent G6410A Triple Quad mass spectrometer in multiple reaction monitoring mode (MRM) using a positive electrospray ionization. The mass transitions monitored were m/z 239 → 127 for rufinamide and m/z 251 → 108 for lacosamide. Sample preparation consisted of L. Theµsimple protein precipitation with methanol, sample required only 50 concentration range was 40 - 2000 ng/mL for rufinamide and 22 – 1100 ng/mL for lacosamide in spiked plasma. The limit of detection was 1.25 ng/mL and the lower limit of quantification was established at 5 ng/mL for rufinamide and 0.7 ng/mL LOD and 2.75 ng/mL LLOQ for lacosamide. A validation was carried out to prove the selectivity, precision, accuracy and stability of the assay, and the matrix effect of different biological fluids, which is needed to justify its use in preclinical pharmacokinetical studies. Short-term –, post-preparative –, freeze-thaw- and working solution stability was also investigated.

Results and conclusions: The proposed method provides accuracy, precision and high-throughput (short runtime – 4.5 min) for quantitative determination of the specified antiepileptics. This is the first reported LC-MS/MS method for analysis of rufinamide from plasma samples, showing higher sensitivity without complex sample preparation. The described method will be applied to accurately measure antiepileptic's concentration in biological fluid samples from preclinical pharmacokinetic studies, but can also be used in clinical studies or therapeutic drug monitoring.

Design, syntheses and biological evaluation of novel DNA gyrase B inhibitors inspired by marine natural compounds

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Bacterial DNA gyrase (Gyr) is the target of many antibacterial agents, e.g. quinolones [1]. The enzyme is present in prokaryotes and some eukaryotes, like malaria parasite Plasmodium falciparum. Gyrase is not present in humans, which makes it a promising target for developing novel antibacterial drugs. Prokaryotic gyrase is a heterodimer of two subunits (A2B2). Gyrase B contains an ATPase domain, while the gyrase A subunit contains a DNA cleavage core. The aim of our study was to design, synthesize and biologically evaluate synthetically accessible GyrB inhibitors, analogs of oroidin, which is one of the bromopyrrole alkaloids from sponges of the genus Agelas. Computer-aided drug design was used for the selection of candidates for synthesis, which was partly based on published procedures [2]. The designed and synthesized oroidin analogs were evaluated in vitro for their inhibition of DNA gyrase B from Escherichia coli, as described [1]. We have designed, synthesized and biologically evaluated several analogs of alkaloids of the genus Agelas that are based on the (S)/(R) 4,5,6,7-tetrahydrobenzo[d]thiazole-2,6-diamine and benzo[d]thiazole-2,6-diamine heterocyclic cores, which were coupled with different activated carboxylic acid derivatives to investigate the impact of L-Pro, pyrrole, bromopyrrole, dibromopyrrole, dichloropyrrole and indole fragments on the E. coli GyrB inhibition. Some of these compounds inhibited GyrB with IC50 values in the low micromolar range. Further structural optimization of the most potent compounds from the first series was accomplished after the acylation of the 2-amine group of 4,5,6,7-tetrahydrobenzo[d]thiazole-2,6-diamine with substituents of different length containing a terminal carboxylic acid group or its ester. In vitro assays on the isolated enzyme showed that these structurally optimized dibromopyrrole-based oroidin analogs demonstrated IC50 values between 62 and 158 nM. Their antibacterial activity is currently under investigation.

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The anthraquinone components of Rubia tinctorum L. are suitable anticancer drugs for application in targeted tumor therapy

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The application of drug delivery systems containing synthetic anthraquinones (e.g. doxorubicin) proved to be efficient in tumor therapy to diminish the side effects and increase the selective cytotoxicity of the conjugated drugs. The alizarin and purpurin – di- and trihydroxyanthraquinones of Rubia tinctorium – were also reported to have antitumor effect. Our objectives were to study the (i) antitumor activity, (ii) the cell adhesion modulator and (iii) chemotactic effects of alizarin, pupurin and an extract of Rubia tinctorum as well as (iv) to analytically characterize this extract in order to find an ideal anthraquinone for design drug delivery systems. The aqueous extract was prepared from R. tinctorum hairy root culture with distillated water for 24 h. The qualitative analysis of the extract was achieved by HPLC and ESI-MS. The cytotoxicity of anthraquinones was evaluated by MTT-assay after 24, 48, 72 h of incubation in two metastatic melanoma cell lines (A2058, HT-168/M1). The effect of anthraquinones on cell adhesion was measured by xCELLigence SP and the NeuroProbe® chamber was used for the chemotaxis assay. According to our results the concentration dependent cytotoxic effects of purpurin was more significant in A2058 than in HT-168/M1 cells, while the alizarin elicited a proliferation inducer activity in both model cells. Regarding the cell adhesion the purpurin had a positive effect in A2058 cells at 10-5 M, while the adhesion of HT-168/M1 cells was decreased at 10-7 M. The extract proved to be more cytotoxic than the pure compounds in equimolar concentrations, but failed to affect the melanoma cell adhesion. All of the tested substances elicited chemorepellent effect in A2058. In the extract, the munjistin was identified as the main component. In conclusions, the significant antitumor activity and chemorepellent effects of Rubia-derived anthraquinones with moiety suitable for conjugation enable them to act as anticancer agents in targeted tumor therapy.

Investigation of the crystallinity of meloxicam co-ground with PVA

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Keywords: meloxicam, polyvinyl alcohol, crystallinity, co-grinding, XRPD, DSC.

Particle size reduction to the submicron region during a grinding process needs a high energy input. This grinding energy requirement can be decreased by suitable additives, e.g. polymer, and performing a co-grinding process. Although these excipients promote attainment of the nanoparticle size range, they can also decrease the crystallinity of the ground active pharmaceutical ingredient (API). The changed crystalline state can cause various problems such as harder processability, instability, different dissolution profile, etc. Some additives have higher ability to decrease the crystallinity of a given drug, some of them have no effect on this at all.

To investigate one of these interactions between a poor glass former API and a polymeric excipient, meloxicam and polyvinyl alcohol (PVA) were chosen to co-grind. The crystallinity change was investigated by XRPD and DSC methods. Crystallinity degree calculations were based on different values of XRPD diffractogram and DSC curve. During the co-milling process the crystallinity of the MX is decreased continuously by the elapsed grinding time. At the end small amount of crystalline drug could be found in the ground media.

Towards the total block of HIV-1 Reverse Transcriptase: the dual-inhibitors approach

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The design of multiple-acting ligands has become a fascinating challenge for the therapy of diseases with multifarious pathological mechanism such as human immunodeficiency virus (HIV-1) acquired immunodeficiency syndrome (AIDS) [1]. The inhibition of multiple targets with a single molecule, in fact, could improve patients' compliance and reduce the occurrence of drug resistance [2]. More than 20 antiretroviral drugs, targeting different steps of the HIV replication cycle, have been approved for the clinical treatment of HIV infected patients [3]. Among these, one of the most attracting and explored targets is the

HIV-1 RT which is responsible for retrotranscription. Currently, two classes of RT inhibitors (RTIs) are included in the approved combination treatments used for HIV-1 handling, namely Nucleoside/Nucleotide RT Inhibitors (NRTIs/NtRTIs) and Non-Nucleoside RT Inhibitors (NNRTIs). However, no drugs are clinically available for the inhibition of the RT associated RNase H function. Most of the RNase H inhibitors identified so far chelate the divalent metal ions (Mg2+) coordinated in the active site, but lack of specific binding [4,5]. Indeed, the development of compounds inhibiting both RT associated RNA-dependent DNA polymerase (RDDP) and RNase H activities would have several advantages, leading to a complete block of RT functions, new favourable drug resistance profiles, reduction of combined drugs and of toxic side effects. In this respect we wish to report the results of our research towards the identification of new small molecules capable to completely block the RT enzymatic activity by the simultaneous inhibition of the two RT associated functions.

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The complete microspeciation of ovothiol, the smallest tetrafunctional biomolecule

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Thiol-disulfide equilibria in amino acids, peptides and proteins (e.g. cysteine, glutathione, oxytocin, vasopressin, somatostatin, insulin, N-acetylcysteine) are essential in maintaining the redox balance, protecting thus the living organisms against oxidative stress. To the best of our knowledge, the smallest, yet most-faceted thiol-contating biomolecule is ovothiol, which occurs as three differently methylated derivatives at the amino group. Ovothiol A, B, and C are mercaptohistidine derivatives first observed in marine invertebrate eggs. These molecules are among the most potent antioxidants found in nature, due to their remarkably low thiolate basicity. Although the redox and acid-base equilibria of ovothiol A have been described, the site-specific characteriziation of this small molecule with as many as four basic moieties (thiolate, carboxylate, amino and imidazole nitrogen) is yet to be elucidated. A deductive method was designed in order to elucidate the entire microspeciation of ovothiol A, by examining three derivative compounds (ovothiol A amide, S-methyl ovothiol A, S-methyl ovothiol A amide) that model the minor microspecies of ovothiol A. The

synthesis of these model compounds was described for the first time. By conducting NMR-pH titrations followed by regression analysis, the macroscopic protonation constants of the model compounds were determined, which in turn were used to calculate the microscopic protonation constants of ovothiol A. Our results include the entire microspeciation scheme of ovothiol A (32 site-specific protonation constants for 16 microspecies) calculated by two independent methods using 1H NMR-pH titration data, these calculations were confirmed with UV-pH titration and 15N NMR-pH titration data. We also determined the interactivity parameters between the proton-binding sites of ovothiol A, along with the standard chemical shift change values of all the nuclei in or near the basic centers.

Chemical analysis and possible application of Nostoc sp. from Hortobágy

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Today most of the drug molecules are derived from plants, fungi or bacteria. Algae or cyanobaceria are having nutritional usage and are potential source of new biologically active metabolites which are only being discovered in the last few decades. Nostoc sp. is one of the well known cyanobacterial (former blue-green algae) species belonging to prokaryotes. These ancient, photosynthetic bacteria can be found all over the world in freshwaters just like in salt waters or even in temporary wetlands. Nostoc species form filaments in microscopic view, but can exhibit different macroscopic shapes according to the habitat. Nostoc is an edible alga, mainly consumed in South-America and Asia. It is rich in proteins, fiber, polysaccharides and trace elements. Moreover it can contain carotenoids, phycocyanin and many other biologically active metabolites. It is capable to produce toxins (microcystine, beta-ethylamino-l-alanine). In recent in vitro and in vivo studies it showed anti-inflammatory, hypocholesterolemic, anti-cancer and antiviral effects. In 2010 we isolated a Nostoc species in Hortobagy, Hungary. Since then we successfully set and sustain a laboratory colony and producing significant biomass. We determined the polysaccharide composition with GC-MS. The main components are xylose, arabinose, mannose and galactose. Carotenoid analysis was also performed with HPLC and indicated high amount of echinenone, beta-carotene and cantaxanthine. Recently we performed feeding tests on rats which showed significant weight gaining without side effects. Samples are still being analyzed. Since this Nostoc species is unique our plan for the future is to show biological effect in vitro and in vivo, hopefully connected to one or more constituent of the alga and to prove the safety of consumption.

Liposomes for triiodothyronine delivery to hepatoma cells: development, characterization and in vitro studies

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Triiodothyronine, T3, is a thyroid hormone that plays an essential role in morphogenesis and differentiation through interaction with its nuclear receptors (TRs). In addition, there is increasing evidence for their role in tumor suppression. The TRs are able, in fact, to inhibit cellular transformation, tumor development and metastatic growth. In particular, although the T3 stimulates the proliferation of normal hepatocytes, it induces a rapid regression of hepatic nodules and reduces the incidence of both hepatocellular carcinoma and lung metastases in rats. However, after T3 administration, significant side effects may also occur. Given the T3 anticancer potential in the treatment of hepatocellular carcinoma, the aim of this work was to develop different liposomes formulations for T3 targeting to liver cancer cells, thus, reducing side effects. Liposomes were prepared according to the film hydration method. T3 content in liposomes was determined by HPLC. Conventional and stealth liposomes were characterized by size distribution, zeta potential and encapsulation efficiency. Morphology of formulations was investigated using TEM. Stability was evaluated on storage at 4ºC for 30 days. In vitro cytotoxicity studies in rat FaO, human HepG2 and SKHep hepatoma cell lines were carried out; viability of FaO, HepG2 and SKHep cells incubated with empty formulations was evaluated for 72 h by NRU assay. Results confirmed absence of toxicity in blank formulations and suggest that liposomes may be a suitable carrier for T3 delivery.

Radiolabeled rhTSH peptide analog for cancer imaging in spontaneous diseased animal models

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Background: The aim of this work was to evaluate the imaging effect of 99mTc-rhTSH in spontaneous diseased animal models. Spontaneous tumours in dogs and cats share a wide variety of epidemiological, biological, and clinical features with human cancer. High

degree of homology can be detected in expression of cell surface antigens and in the case of various signal transduction pathways. These are the factors that contribute to the advantages of the companion animals as a model for human neoplastic diseases.

Materials and methods: In our whole body 3D SPECT/CT examinations we used altogether 4 referred dog and 2 cat patients: 3 dog patients with thyroid cancer, 2 cats and 1 dog having other type of head and neck cancers. We labelled an aliquot of recombinant human Thyroid-stimulating hormone (rhTSH) with 925 MBq 99mTcO4 in tricine buffer. To avoid the unnecessary degradation of image quality caused by unbound pertechnetate we performed purification with a PD 10 gel chromatography column. Labelling efficiency was determined with ITLC-SG in 0,9% NaCl solution mobile phase.

Results: The rhTSH analog showed high specific uptake in two dogs with thyroid carcinoma but in the third dog which had tumour resection before the examination we saw inhomogeneous uptake. The cervical region of the operated area 2.4X3.2 cm diameter primary thyroid tumour residuum displayed correctly. We saw 4 cervical and 1 mediastinal lymph node metastases and in the lungs several tumor metastasis (2-4 mm diameter) were found. Only low, non-specific uptake was detected in the two cat and one dog patients with other head and neck cancers. The animals tolerated the radiopharmaceutical applications well; neither acute nor chronic side-effects were detected.

Conclusion: 99mTc-rhTSH analog seems a promising probe for molecular imaging in companion animals with different spontaneously occurring tumours which are overexpressing TSH receptors. Scientific work was supported by several national (KMOP-1.1.1-08/1-2008-0017, KMOP-1.1.1-09/1-2009-0056, GOP-1.1.1.-09/1-2010-0107) and international projects (IAEA-CRPs, EMIL NoE).

The induction of in vitro tissue cultures and the study of alkaloid production in Galanthus species

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The Amaryllidaceae family is characterized by the occurrence of species-specific alkaloids usually with a backbone containing 15 carbon atoms. In addition a number of compounds are highly toxic alkaloids known to be pharmacologically beneficial, such as galantamine. The principal aim of this study was to develop an efficient in vitro plant regeneration method suitable for germplasm preservation in Galanthus nivalis, Galanthus

elwesii, Galanthus woronowii and later examination of their alkaloid content. The tests were made using thin layer chromatography (TLC) and HPLC analytical methods. G. nivalis (snowdrop) is a popular late winter- early spring flowering geophyte belonging to Amaryllidaceae. The type of auxin is of key importance: high concentrations of α-naphthalene-acetic acid (NAA) proved to be the most suitable for G. nivalis cultures. Culture media contained a cytokinin (benzyladenine, BA) as well. We have proved that a relatively high auxin and low cytokinin concentration is required for whole plant regeneration on bulb scale derived calli. Histological analysis of cultures capable of plant regeneration revealed the presence of early stages of somatic embryo development after 42 days of subculture. Thus, cultures able of regeneration were embryogenic. Calli were initiated on MS medium with either 2 mg l-1 NAA and 1 mg l-1 BA or 10 mg l-1 NAA and 1 mg l-1 BA– that is, auxin/ cytokinin ratio was relatively high. Galanthus elwesii and Galanthus woronowii tissue culture could be successfully obtained, but bulb regeneration was achieved in the presence of indole-acetic acid (IAA) and NAA. TLC and HPLC chromatograms identified galantamine and licorine in all three tissue culture samples (callus, shoot, bulb) of G. nivalis. Thus, a well established G. nivalis G. elwesii and G. woronowii tissue culture may have an importance in species conservation as well as biotechnology.

POSTER PRESENTATIONS – BIONICS

Development of a symmetric single-plane illumination microscope

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To better understand development of complex biological systems, imaging of living samples over time is necessary. Using the light-sheet based Single-Plane Illumination Microscopy (SPIM), it became possible to image live specimens with high resolutions in 3 dimensions over time. Fast image acquisitions makes cell tracking possible even in highly motile samples, such as a developing drosophila embryo, zebrafish brain, or phallusia embryo. This microscopy technique uses two perpendicularly aligned objectives. One is used to illuminate a thin plane of the specimen, and the other is used to capture an image of this plane. Compared to confocal microscopy, this method provides better 3D resolution, higher acquisition rate and less phototoxicity. This makes light-sheet based microscopy especially suitable for imaging live specimens over a long period of time. The aim of this work is to build a SPIM that uses identical objectives, and uses both of them for illumination and detection in an alternating fashion. This will significantly improve 3D resolution, because the two images taken from different views can be fused together providing a better resolution than either of them. To be able to control and automate the microscope, we also designed a custom program that controls image acquisition and handles user interactions. Our aim when developing this program was that it should be modular and easily adaptable to different kinds of SPIM setups. Our final goal is to use this microscope to image live specimens. Using data acquired with this microscope it will be possible to segment cells, and track cell divisions and cell migrations. This will provide valuable information on embryonic development in several species.

In vivo testing of silicon based neural multielectrodes with simultaneous drug delivery

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Stimulation and recording of the neuronal network is probably the most widely used method in experiments to understand the function of the central nervous system. A quite new approach in the stimulation of the brain besides electrical excitation is injecting functionally relevant molecules directly into specified areas of the brain. The technique is called chemical stimulation. Since the treatment of brain with drugs is not trivial because of the blood-brain barrier, a tool for chemical excitation could also be used for clinical applications in problems such as brain tumor therapy. Si based technology is capable of creating needles in the range of micrometers with monolithically integrated microfluidic channel within. With such a device invasive measurements and fluid delivery can be done with relatively high spatial accuracy and small tissue damage. In this work, the fabrication method, fluidic characterization and in vivo testing of the first deep brain silicon multielectrode with monolithically integrated fluidic channel are presented. Micromachined silicon probes with monolithically integrated microfluidic channels up to 70 mm length have been realized to perform simultaneous electrical recording and drug delivery in deep brain regions. The fluidic microchannels are geometrically characterised by cross sectional SEM imaging, cross section of the microchannels is in the range of 5-30 um, while the length of the channel can be even 70 mm long. The functionality of the microfluidic channels is verified and the hydrodynamic characteristics (flow rate vs. injection pressure) are measured in the case of several length and cross-sections. Feasibility of our integration concept is proved by locally injected bicuculline in the cortex and in the thalamical regions of rat brain in vivo, while simultaneously recording the electrical signals of the stimulated neurons on four different electrical channels.

When bio-Nanotechnology meets Microelectronics

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During the last years, material science was focusing on the exploration of the material characteristics at nanoscale. To completely exploit the ultra-small dimension and high sensitivity of these materials, researchers addressed the development of nanodevices including only a single nanostructured element, such as nanowires (NWs), nanotubes, molecules or nanoparticles. These nanomaterials can also be considered the basis for a new generation of bio-sensors able to interact with gas, molecules (e.g., DNA molecules) or other bio-substances at nanoscale. To electrically connect the nano-element, we use planar gold nanogaps (<10nm) organized in arrays and obtained through electromigration process controlled by a full custom PCB-based modular system. During first experiments, monolayers of conductive Thiophene molecules have been self-assembled onto the nanogap resulting in a gold-molecules-gold molecular junction. Even functionalized NWs can be placed in the nanogap using dielectrophoresis. The I/V characteristic of a Metal-Molecular-Metal junction shows that a plausible resistance is in the range 10MΩ – 10GΩ but it strongly depends on the size of NWs or on the type, the number and the length of bonded molecules on the nanogap. Basically, these new generation sensors rely on changes of electrical properties (R, C) of nanodevices that have to be converted into electrical signals with an ad-hoc interfacing circuit fabricated in a standard low-cost technology. The CMOS process satisfies these requirements. The design of the read-out circuit has to garantee: – large R and C read-out range, due to process variation of nanodevices; – high SNR, to measure ultra-low current flowing through molecular nanodevices; – low power consumption to support high densitity integration of nanosensors organized in array; A quasi-digital Resistance to time-domain converter (e.g., Resistance-to-Frequency(R2F), Resistance-to-Time(R2T), PWM) can be an adaptive and effective solution. The proposed R2F converter shows low measurement error (<1%) within the 50kΩ – 3GΩ range and consumes 142μW [1]. Moreover, the last R2T prototype consumes only 8.5μW, it has higher linearity in the whole range with maximum measurement error of 0.8%.

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Models and biomaterials for ageing bone tissue regeneration

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In the last few decades, as a result of decreasing mortality and birth rates in Europe, the population has steadily aged and thus the scientific community interest on pathologies associated to aging has raised. Aged bone is characterized by loss of bone mass and micro-architectural skeletal deterioration which compromise its response to pathological conditions, trauma and environmental factors. This work aims to overcome the current lack of suitable models for the study of physiological conditions associated with bone aging processes, in order to support the design of new regenerative approaches of the associated pathologies such as osteoporosis or. Specifically, this research aims at creating composite scaffolds, characterized by different composition, physical-chemical and mechanical properties, that will serve as models of the healthy and aged bone tissue, once they have been colonised by cells. For this purpose, a bioactive glass (CEL2) was produced and then surface-coated with a biosynthetic blend based on type I collagen (coll) and a water soluble polyurethane (PU) to mimick bone-ECM structure. The glass belongs to the system SiO2–P2O5–CaO–MgO–Na2O–K2O. Type I collagen was isolated from femurs and tibias of adult New Zealand rabbits. PU was synthesized from polyethylene glycol, 1,6-esamethylen diisocyanate and N-BOC-Serinol. Glass samples were superficially silanized by using a 2% triethoxysilane solution, for further genipin (GP) grafting (5mg/mol). GP is then used as crosslinking agent for coll. Two different coll-PU blends, without (A) and with GP (B), were evaluated. Such blends were tested at different coll concentrations. Surfaces modified with coll-PU blends demonstrated high hydrophilicity. Moreover, high PU content enhanced the surface wettability. CEL2 scaffolds were successfully functionalized with biopolymeric blends. The use of GP as crosslinker in the blends allowed incorporating a higher amount of collagen on the surface. Blends with higher concentration of collagen evidenced a more suitable contact angle for cells interaction.

A Mathematical Framework for the Analysis and Modelling of Memristors

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The memristor constitutes a two-terminal fundamental passive circuit element originally theorised in 1971 by Leon Chua. In 2008 HP fabricated for the first time nanoscale memristors. The memristor's recent rediscovery by HP attracted the attention of the scientific community and the technology industry due to its many potential applications. The attractive properties of memristive devices (e.g. nano-scale dimensions, non-volatile memory, low-power consumption and compatibility with CMOS to name a few) make these devices ideal candidates for improving the performance of conventional computer memories, prolonging the lifetime of the CMOS technology (which is reaching its physical limits) and enabling the implementation of large-scale adaptive/learning circuits. Despite the considerable progress in the field, a clear challenge still remains the articulation of a general mathematical framework for facilitating the analysis and modelling of memristors complying with Chua's 1971 definition. A first step in this direction has been made by our recent work which shows that memristors' output dynamics comply with Bernoulli (BDE) and Linear (LDE) differential equation. The identification of these dynamics allows us to define a set of formally solvable general solutions for the output response of all types of ideal memristors. By applying the appropriate general solution we are able to evaluate analytically the output response for HP's memristor model. Moreover, by exploiting the analytical output response of HP's model we have studied several device properties of memristors such as their hysteresis, harmonic distortion and their behaviour in series and parallel networks. The insights gained through our analytical approach can also be useful when modelling practical memristive devices.

Analysis of the amyloid-forming proteins

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The diseased aggregation of proteins, in which the originally globular or unstructured proteins are forming stable, resistive polymer fibres through a temporary structure, is called amyloidosis. The deposits of amyloid plaque are polymers of misfolded proteins, which typically have antiparallel beta-sheet structure. There are many known diseases

(e.g. Alzheimer's, Huntington's, Parkinson's and Creutzfeld-Jacob diseases), where the development of the clinical picture is related to the deposits of these protein aggregates, and the forming of plaques. Because of the better knowledge about the structural background of amyloidosis, the sufficient number of experimentally proven amyloid forming proteins, and the more and more reliable amyloid prediction algorithms, we analyzed the amyloid forming sequences and the so called flanking regions around them. During my work, I searched for properties of the sequences that enhance or inhibit the amyloid formation. All experiments were based on the primary structure of the protein, and the physical and chemical properties of the amino acids. Our results contribute to the better knowledge of amyloid formation, and the better understanding of the genetic background of diseases connected to amyloidosis. My job is also the part of a larger project, in which we try to have a better understanding about the regulation of intracellular protein-protein interactions, and how to avoid the amyloid formation of such interaction sites.

Analysis of maternal–fetal heart rate interaction

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Coupling of the maternal–fetal heart showing a complex dynamical behavior is a good example for the interaction of integrated physiological systems through neural regulation. By investigating the maternal and fetal heart rate variability we researched the coupling of these two indirectly connected systems considering such basic physiological phenomena as the progression and the operation of the fetal heart and the maternal cardiovascular or respiratory changes. To verify the assumptions we recorded CTG-measurements with a non-invasive phonocardiography based device using the classical (non-stress) and a special own designed protocol. During the data processing, we concentrated on the accurate determination of the fetal and maternal heart rate values, the frequency spectrum of the heart rate variability and additional derived parameters. We can observe that the fetal average beat-to-beat time decreased significantly ($p<0.05$) as the physical load increased on the mother which was also reflected by the decreased maternal beat-to-beat times. In addition, the indices of the activity of the fetal autonomic nervous system changed according to the expected tendencies. Furthermore, using a specialized stroboscopic technique we managed to identify synchronization epochs reflecting different fetal–maternal heart rate ratios. The mapping of these relationships may also be relevant in the clinical practice since in this way new information may be acquired for the monitoring of the fetal well-being.

Supervised and unsupervised learning from EEG and fMRI data for supporting presurgical evaluation in refractory focal epilepsy

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Approximately 30% of all epilepsy patients suffer from refractory seizures despite anti-epileptic drug therapy. Resective epilepsy surgery can offer a remedy in these cases. A key to successful surgery is a rigorous multimodal presurgical evaluation localizing the epileptogenic zone (EZ). This work aims at improving two independent aspects in the existing procedure. First, we present a novel patient-specific seizure detection algorithm exploiting structural information from the inherently multidimensional EEG signals using nuclear norm regularization. We prove that the proposed algorithm achieves successful seizure detection requiring a reduced number of training seizure examples compared to traditional approaches. Early seizure detection facilitates reliable localization of the EZ using ictal SPECT co-registered to MRI (SISCOM). Alternatively, simultaneous EEG-fMRI, analyzed with the general linear model (GLM), can provide accurate localization of hemodynamic changes corresponding to interictal discharges observed on EEG. However, a majority of EEG-fMRI studies fail due to the lack of visible interictal spikes. Therefore, we use independent component analysis (ICA) of fMRI to reveal sources related to the epileptic network. We show that the epileptic independent components overlap significantly better with the EZ, than activation maps obtained by GLM-based EEG-fMRI analysis do. Moreover, we show that sources related to the epileptic network are found with ICA even in patients in whom no interictal spikes could be identified in the EEG. Therefore, we conclude that ICA has a great potential in extending the applicability of interictal fMRI recordings and could contribute to presurgical evaluation in the future.

Simulation-based investigation of the effect of two-photon microscope operating parameters on the image quality and photobleaching

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Two-photon microscope became widely used in biomedical research due to its high axial resolution, deep penetration, and lower photobleaching rate compared to one-photon excitation confocal microscope that enables even in vivo imaging. However, photobleaching (i.e. the irreversible breakdown of fluorophore molecules) is still a limiting

factor of the image quality and the duration of examination. In this computational study the number and spatial distribution of detected fluorescent photons and photobleaching processes are investigated in case of different microscope settings (e.g. power, pulse length, pulse shape, and pulse repetition rate of illuminating laser) and fluorophore photophysical parameters. The simulator program is based on a three-level (ground state, excited state, triplet state) photodynamic model and includes four photobleaching pathways (photobleaching by one and two-photon excitation from both excited and triplet state). The sample is modeled as a homogenous fluorophore solution that is divided into cubic volume elements. The simulator computes the rate of state transitions and the number of molecules per state in each laser cycle, for each volume element. The diffusion of fluorophore molecules is also simulated. Illumination is considered to be fixed to one focal point and it is described as a Gaussian beam. Simulation results for different example fluorophores are presented to illustrate both temporal and spatial characteristics of photodynamic processes in two-photon microscope as a function of its operating parameters.

Flexible MEMS-based microelectrocorticographs (uECoG)

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Electrocorticography (ECoG) or intracranial EEG can be employed to measure the local field potential (LFP). Significant modulations in LFP are related to sensory processing, motor planning, visuomotor interactions and higher cognitive functions such as attention, memory and decision-making. Electrocorticography is also used to locate epilepsy seizure focus areas, before surgery. EcoGs used in clinical practice have an electrode diameter of 1 mm, which provide insufficient spatial resolution when investigating brain phenomena. In this work microelectrocorticograms are realised using MEMS technology, enabling the fabrication of electrode sites on the order of 100 microns. The devices feature a polyimide structural and SU-8 passivation layer, with gold used as the material for the electrodes and wiring. The materials used make the device flexible, which is mandatory for chronic implantation. We present the technological process, which improves upon the previous polyimide-based devices in manufacturing considerations. Microelectrocorticograms were designed specifically for chronic implantation into rat and feline cortices, and feature micromachined holes, which help the circulation and prevent damage to the animal. The electrical connections were realised in a compact way, with both solderable preciDIP-24

and mechanical contact ZIF connectors. The manufactured uECoGs were characterised using impedance spectroscopy, including a one week water leak test, where the electrodes were submerged in a saline solution (Ringer TEVA). The characteristic impedance of the channels were found to be on the order 10 kΩs at 1 kHz. Preliminary in vivo testing was carried out in an anesthetized Wistar rat, where the uECoG grid was placed onto the intact surface of the brain. Slow-wave oscillations characteristic of the anesthesia were recorded.

Effect of scatterer density on the lateral spatial frequency spectrum in ultrasound images

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In clinical ultrasound imaging, it is necessary to know the sound of speed in tissue to produce an image with sufficient quality. A method for determining the sound speed in a medium is by generating images with multiple sound speeds, and choosing the one as optimal that is the sharpest. The sharpness measurement most often used is based on integration of the lateral frequency spectrum of the image. In both simulation and real data, experience showed that the sharpness measurement based on spatial frequency becomes unreliable once certain parameters in the medium change. Specifically, increasing the number of scatterers causes the maximum sharpness measurement to shift towards lower sound speeds, although the acoustic properties (such as the sound speed) supposedly remain unchanged. The observed phenomenon was the initial motivation to study the lateral frequency spectra of images generated by different sound speeds. Our research had the following aims: understanding the origin of the observed phenomenon, finding a connection between the spectrum and structure that is quantifiable, and possibly can have clinical applications. We concluded that the dependence of the occurrence on scatterer density implies that the main reason for it is the interference of the scatterers' point spread functions. Therefore, in a simplified model, the lateral frequency distribution of two scatterers was simulated, with varying distance between them. Data showed that by placing our scatterers at different distances from each other, we can determine the lateral and axial distance which causes the distortion of the original (one scatterer) distribution. Data suggests that the lateral frequency distribution correlates closely with structure, especially mean distance between the scatterers. A mathematical solution that more closely describes the relationship is yet to be found. If such a solution can be implemented, it may mean an easy, non-invasive, painless, cost-effective way of characterizing tissue in vivo, and as such become a powerful diagnostic tool.

High-resolution structure and actin-binding of synthetic myosin thick filaments

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The most important tissue of macroscopic biological motion is the striated muscle, in which actin filaments interact with myosin II motor proteins organized into bipolar thick filaments. Although it has been shown that thick filaments polymerize in an ionic-strength-dependent manner, their exact structure and the underlying molecular mechanism are unknown. According to most muscle contraction models, these polymers stochastically interact with actin filaments. However, the details of this interaction are not fully understood. We examined the structure of synthetic thick filaments (TFs) and their interaction with actin filaments by using atomic force microscopy (AFM), which is capable of high-resolution imaging under aqueous conditions. Thick filaments were synthesized from rabbit back muscle myosin with dialysis at different ionic strengths. The TFs, together with rhodamine-phalloidin labelled F-actin, were adsorbed to mica surface in buffer, their topography scanned with AFM and the fluorescent signal examined with total internal reflection fluorescence (TIRF) microscopy. While on mica, thick filaments displayed an opened-up arrangement, enabling us to observe the details of the internal structure, such as subfilaments of the shaft, which consisted of helically entwined tail domains. The structural compactness of the TFs depended on ionic strength, as the average width was 103+-23, 179+-34, 167+-38, 162+-43, 159+-41 nm at 0, 30, 60, 90 and 120 mM KCl, respectively. In the axis and the middle of the filaments at 0 mM KCl a solid core was observed, which contained 2-3 subfilaments. At higher ionic strength we could resolve to 3-10 thinner subfilaments branching out into individual myosin tails by the ends of the filament. Actin filaments did not bind directly to the surface, but the presence of myosin fixed their position, and in both TIRF and AFM images they were observable. Their interaction with individual and filamentous myosin molecules could be examined. Our results reveal details of thick filament internal structure not described for vertebrate myosin before. Although many structural details, for example the mechanism of bipolarity, need to be clarified, the arrangement of myosin molecules in thick filaments is such that the motor domains at their surface can optimally attach to actin filaments.

Integrated microcapillary system for microfluidic parasite analysis

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A microfluidic separator device has been designed and fabricated to detect micron-size parasites from blood samples using a monolithic polydimethylsiloxane (PDMS) microfluidic structure. Several intravenous parasitosis can be observed by the constructed integrated microcapillary system such as dirofilariasis or Lyme disease. [1,2,3] Inside the developed Lab-on-a-chip (LOC) device a kidney-shaped, flow-through microfluidic separator structure has been implemented. The diameter of the cylindrical Active Zone (AZ) is 1 mm, where the larvae of nematodes or other parasitic infection remain trapped. The AZ region is partially surrounded by rectangular cross-section shaped flow-through microcapillaries. 48 different microfluidic devices have been designed, fabricated and tested with similar geometry and structure by varying the width of microcapillaries between 6.1 μm and 83.6 μm. The channel height of the microcapillaries is 20 μm and their length is 100 μm. The capillary channel width is the same within one device, where biological samples can flow through. The tested 48 different devices have different channel widths and the number of microchannels is adjusted accordingly. In this case the developed test can be optimized for a specific nematode or parasite detection by changing the capillary width. The velocity and pressure profiles of the developed parasite trapping microseparator has been geometrically optimized by Comsol Multiphysics. The time-dependent incompressible Navier-Stokes equations were solved by Computational Fluid Dynamics (CFD) solvers with particle tracing tests to simulate trapping effect.

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Synchronization in Networks of non-identical Phase Oscillators

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Synchronization, as a well studied cooperative behavior, is observed in both natural systems like coordinated activity of heart cells and collective flashing of fireflies, but also in man-made systems such as oscillator arrays, lasers and chaotic circuits. In real systems, nodes are not identical and communicate through non-identical interactions. A particular interesting phenomenon happens in a population of phase oscillators with arbitrary distribution of natural frequencies where synchrony occurs even though the oscillators' interactions are not identical. In this work we study such networks of phase oscillators with arbitrary topology using a generalized Kuramoto model and we show that the frequency synchronization manifold is stable under some constraints on the interactions. We consider several example systems with different type of interactions between the oscillators using linear, cubic and sinusoidal periodic coupling functions. By numerical simulations we confirm the analytic findings and further study some phenomena such as phase clustering in a strongly coupled network of oscillators. We derive an expression for the critical coupling gain which is necessary for synchronization in case of fully connected network of identically and linearly coupled oscillators with arbitrary natural frequency distribution. It was numerically observed that synchronization happens at an exponential rate when the coupling gain is sufficiently strong and in that case all nodes oscillate with a common frequency that is the mean of the natural frequencies. Furthermore, we study systems with frustrated interactions and external fields in the dynamics. The topological effects on the synchronization properties are analyzed using random weighted networks.

Flow-through functionalized PDMS microfluidic device for sandwich ELISA

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In this research I try to design a flow-through functionalized PDMS-glass microfluidic device, which is able to detect specified antigens from blood, wine or urine. The surface of PDMS channel is going to be modified for sandwich enzyme-linked immunosorbent assay (ELISA). For detection I plan to use a spectroscopical method. Three models have been designed and tested in a flow simulation program to choose an optimal geometry for fabrication. The first steps for the microfluidic device fabrication are the designing

and modeling of the microfluidic channel. I created three models in AutoCAD software. All of the models have the same height (20 μm) and width (200 μm). The first model is a simple channel without any additional structures in it. In the other models the channel is divided into three parts, and in the middle area two kinds of objects were placed. I used round structures and squared structures. I tested the channels in Comsol flow simulation software. Running the simulation a velocity profile, pressure profile and a particle tracing profile in the microfluidic channel has been obtained. The three models have three different profiles in all three measured categories. The particle tracing profile shows the particle's trajectories in the channel. This is an important information, because it makes calculable the particles (antibodies) binding probability to a given surface, which is a key attribute for ELISA microfluidic devices. The results of simulation showed, that much more particals stucks onto the surface of round structures. These structures have a kind of geometry, which direct the springing down particals onto the channel wall, where they can bind. In this flow-through microfluidic channel I plan to implement a standard sandwich ELISA method. In this method there are two kinds of antibodies: primary antibody – which is immobilized on the solid surface and binds the antigen from the sample – and the secondary antibody – which is labeled with an enzyme for detection. Through the binding of antigen-antibodies and the conversion of labeling enzyme we can measure the amount of the antigen in the sample quantitatively. During the implementation I would try two different ways: full flow-through and a half flow-through solution. The main difference between them, how long time the different components spend in the channel.

Identification of complex systems by Reaction-Diffusion Cellular Nonlinear Networks (RD-CNN)

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By assuming RD-CNN, the derivation of a time efficient procedure allowing the identification of complex systems is addressed in this contribution. The goal is to determine the weight functions of a RD-CNN model by using Electroencephalogram (EEG) signals in epilepsy. The approach is based on a transformation of RD-CNN state equations into the Laplace domain and on the approximation of nonlinear reaction terms by applying a series expansion. Thereby, the expansion coefficients are obtained in an optimization procedure using Simulated Annealing and Simplex downhill methods. The main advantage of the model representation in the Laplace domain is to obtain a system of coupled algebraic equations allowing a simplified determination of a RD-CNN model

as compared to time domain based methods. The performance of the proposed method was evaluated by assuming a FitzHugh–Nagumo system. A detailed discussion will be given in this contribution.

CytoMimetic Circuits: A Novel, Ultra-Low Power, Nonlinear, Bioinspired Circuit Approach To Cellular & Molecular Dynamics Computation

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A novel category of weak-inversion, log-domain, bioinspired circuits called "CytoMimetic Circuits" is proposed for the computation of nonlinear cellular and molecular dynamics. This novel category of circuits exploits the striking similarities existing between the Nonlinear Bernoulli Cell Formalism (an advanced mathematical framework stemming from the Bernoulli Cell Formalism originally exploited for the modular synthesis and analysis of linear, time-invariant, high dynamic range, logarithmic filters) and coupled nonlinear ordinary differential equation, typically appearing in models of naturally encountered biochemical systems. By transforming the biochemical equations into their electrical analogous equations with the use of the aforementioned mathematical formalism, we are able to convert challenging biochemical reactions into electrical circuits comprised only of basic monolithic elements, i.e. MOS Field Effect Transistors and linear capacitors. As a result, completely tunable, continuous-time, continuous-value, low-power CytoMimetic electronic circuits are generated, which are able to simulate fast and with good accuracy cellular and molecular dynamics that match the input-dependant nonlinear dynamics that take place in cellular/molecular level. A large number of biological systems have been implemented via the proposed CytoMimetic circuits and have been found to be in very good agreement with their biological counterparts. Konstantinos I. Papadimitriou Ph.D student & Research Assistant Bioinspired VLSI Circuits & Systems Group Department of BioEngineering Imperial College London.

Controlling wetting properties of silicon-based digital microfluidics systems

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Lab-on-a-chip (LOC) devices are a new paradigm in biomedical microelectromechanical systems (Bio-MEMS), promising a smaller device size, smaller sample and reagent requirements, better cost efficiency and more rapid sample analysis than current medical diagnostic devices. Furthermore, droplet-based (digital) microfluidics may provide a portable alternative to industrial liquid handling platforms. Based on these facts, in cooperation with the Research Institute for Technical Physics and Material Science (MTA TTK MFA MEMS Lab.) and the Computer and Automation Research Institute (MTA SZTAKI) of the Hungarian Academy of Sciences, development of a LOC device for diagnostic purposes is underway. The phenomenon of electrically controlled wetting was observed on silicon-based chip prototypes. The first successful actuation experiments were performed on thin film microstructures applying a square wave of 60 V amplitude. Higher voltage values did not improve actuation. Based on these results, silicon-based single-plate digital microfluidic chip prototypes were designed and manufactured. Upon application of a square wave of 100 V amplitude, inter-electrode actuation was achieved. The latest design to date is a 10x10 regular array of electrodes capable of x-y actuation of droplets at 30-300 Hz, 80 V. Moreover, a thin film based superhydrophobic surface was implemented and measured with respect to wetting. Such a layer can potentially extend the lifetime of LOC systems by providing additional protection against contamination and decrease the interaction between the sample and surface. Our goal, currently a work in progress, is to implement a closed, programmable, automatic fluid handling LOC device capable of processing up to 6 samples or reagents via external fluidic inputs and outputs. This automatic fluid handling platform is primarily intended as a low-cost fluidic automation solution for sample preparation purposes.

EXG: a simultaneous EMG and EEG recording to study movements from the genesis into the brain to the actuation in the muscle

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Moving a muscle implies the communication between the muscle and the brain. The impulse is generated in the motor cortex and it is carried by the nerves to the motor end plate on the muscle where it stimulates the muscle to contract. To study the phenomenon in a fully comprehensive way then is necessary to evaluate an EXG signal composed not only by the myoelectric signal(EMG) but also by the electroencephalographic(EEG) one. For this reason we've designed two acquisition devices to analyze simultaneously EMG and EEG signal. The muscle activation is recorded using a multichannel Average Threshold Crossing wireless system, based on Willison principle. The device is composed by a trasmitter and a receiver.The trasmitter comprises a signal amplifier, an asynchronous threshold detector and an asynchronous IR-UWB transmitter. The receiver comprises an asynchronous IR-UWB receiver plus a digital logic which implements a windowing algorithm on the received events. To evaluate brain activity we've designed a multi channel EEG acquisition system that removes noise and amplifies the input signal through Butterworth filters. A Right Leg Driver amplifier is employed to improve the common-mode rejection of the device. The eeglab matlab toolbox is used to analyze the raw signal recorded and to draw, using the indipendent components analysis (ICA), a map of the scalp with the different neural activities. The results of the in vivo experiment of the two devices were promising, the signals obtained were good and they reflected correctly brain and muscles activation. The next step will be totally integrate the two devices to obtain an EXG acquisition system. This will made possible to study the movement from the genesis into the brain to the actuation in the muscle.

Models and biomaterials for ageing cardiac tissue regeneration

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The ageing of heart is associated with a lot of structural and functional changes; the most relevant of them are a great loss of cardiomyocytes, post-mitotic cells, and an increase in systolic blood pressure, due to blood vessel age-related modifications1.

Transforming growth factor-β1 expression, that stimulates the production of collagen, increases with pressure overload, while metalloproteinases, responsible for collagen degradation, are down-regulated. This process brings to an increase of type I collagen and collagen cross-linking, thus causing heart fibrosis1. Currently, models able to reproduce the physio-pathological conditions of aged heart lack. Tissue Engineering strategies could be useful to develop in vitro dynamic models simulating ageing hearts, while respecting the three Rs ("Replacement, Refinement, Reduction") of animal testing. In this work, several polyurethane-based scaffolds were fabricated through the Thermally Induced Phase Separation technique to simulate both health young heart and aged heart. The differences between the two models mainly refer to mechanical properties and micro-architecture, having the aged heart model a major rigidity and smaller pore diameter. Different polymers and polymer solution concentrations were used, depending on the degree of ageing to simulate. The mechanical properties of the fabricated scaffolds were investigated through tensile tests, while scanning electron microscopy (SEM) was used to examine their micro-architecture. The elastic modulus of the structures was in the order of a few MPa, with slight differences between the young and aged-heart simulating scaffolds. Pore size was calculated from SEM images and resulted in the range of tens-hundreds of μm. The scaffolds were also biologically characterized by in vitro cell tests, with non-cardiac cells, showing a good biocompatibility. Biological static and dynamic tests with rat cardiomyocytes have been further planned, with the aim to check the suitability of the fabricated scaffolds to model the heart.

The role of disorder in the degradation of mRNAs containing a premature STOP codon

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Nonsense-mediated mRNA decay (NMD) is a cytoplasmic surveillance system that identifies and degrades mRNAs containing premature termination codons (PTC), thus prevents the production of truncated and inappropriate proteins. The mechanism of NMD, especially the basic sign of recognition is different in various taxonomical levels. In yeasts the downstream sequence elements (DSE), in worms, insects and plants the extremely long 3'UTR, while in mammals and also in plants exon-junction protein complexes help the cell to recognize PTC-containing mRNAs. In NMD, large protein complexes cooperate to trigger degradation of mRNA with a PTC. Due to the extreme variation in the size and

topology of its mRNA substrate, the structural and dynamical details of the mechanism are little understood. Based on bioinformatics predictions, we suggest that fly-casting mechanisms enabled by long disordered regions in NMD complexes are needed for the effective long-range communication. In this work our aim was to analyze the sequence, structure and function of proteins in the mechanism, and to compare the three types of mechanisms. To this end, we have examined five well-described species which differ in the NMD-type they use (S. cerevisiae, C. elegans, D. melanogaster, A. thaliana, H. sapiens). The best-known mammalian intron-based NMD was examined in more detail. We observed a very high incidence of long disordered regions in proteins in NMD complexes and in those mediating their interactions. We have also determined that the appropriate orthologues of human NMD-proteins in inferior organisms usually contain as many disordered regions as the human NMD-proteins, which suggests that protein disorder is evolutionary related to the process of nonsense-mediated decay.

Design and comparison of micromixers using COMSOL simulations

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Clinical laboratory tests are performed to detect diseases, pathological cases (blood analysis, immunoassay). However, these tests require laboratory infrastructure, trained technicians, and often expensive equipment. Application of point-of-care (POC) devices and quick tests is becoming more and more frequent and offers new possibilities in medicine. Microfluidics provides integrable, portable diagnostic devices, which require a very small amount of sample. These lab-on-a-chip (LOC) devices are capable of obtaining laboratory tests, are a subset of MEMS devices and often indicated by Micro Total Analysis Systems (μTAS). The right concentration and homogeneity is a key issue in manipulation of biological samples. One of the important features of a microfluidic system that is integrable into bioanalytical devices is the dilution and complete mixing of the analyte with an adequate buffer solution to ensure homogeneous concentration distribution of the species reaching the sensor area. At the range of 10-100 μm, fluid flow is always laminar, which makes mixing difficult. We have to develop new structures which optimize mixing efficiency. The behavior of different mixer structures can be analyzed by numerical modeling and their efficiency determined experimentally. Designing micromixers is a new task in engineering, existing macroscale devices cannot be simply scaled down for microscale use. In this work, we present the results of numerical simulations with different

micromixers that describe their efficiency and disadvantages, and we make suggestions on enhancing the mixers' geometry. We present both visual and numerical methods to describe the goodness of the mixing that enables quantitative comparison of different devices. Based on measurements carried out with a prototype device, we verify the computational model. Our results are valuable in the development of more effective micromixers and LOC devices.