

Faculty of Medicine Taborska ulica 8

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PRESENTATION OF LABORATORIES OF THE FACULTY OF MEDICINE OF THE UNIVERSITY OF MARIBOR





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CENTER FOR HUMAN MOLECULAR GENETICS AND PHARMACOGENOMICS & DEPARTMENT FOR BIOCHEMISTRY AND NUTRITION

HEAD OF DEPARTMENT Full Prof. Uroš Potočnik, PhD

TEAM

Asist. Prof. Katja Repnik, PhD Asist. Prof. Helena Čelešnik, PhD Asist. Mario Gorenjak, PhD Asist. Matjaž Deželak, PhD Asist. Prof. Vojko Berce, PhD Asist. Staša Jurgec, BSc Carina Pinto Kozmus, MSc., PhD student Larisa Zemljič, BSc., PhD student Gregor Jezernik, MSc., PhD student



Team and head of department

LIST AND DESCRIPTION OF LABORATORY EQUIPMENT

1. Next generation sequencing system for DNA sequence analysis (Illumina MiSeq) enables genomewide analysis with a capacity of 15Gbp in one run. The system is capable of simultaneous in a single experiment diagnosis of most rare hereditary diseases and detection of novel rare DNA polymorphisms that act as genetic risk factors for chronic disease pathogenesis. It also enables the quantification of gene expression and detection of new RNA transcriptions. 2. Real-time PCR system and digital PCR (Life Technologies QuantStudio 12K Flex) is used for effective genotyping of DNA single nucleotide polymorphisms (SNP) using the Taqman method (up to 110,000 genotypes per day) and detection of somatic mutations in cancer. The system is used for gene expression analysis and ncRNA profiling such as miRNA.

REASEARCH INTERESTS AND POTENTIAL FOR COLLABORATION

- Genetic risk factors (susceptibility to complex diseases)
- Molecular mechanisms of disease pathogenesis
- Molecular targets for development of new generation of biological drugs
- Molecular diagnostics including diseases subtypes
- Prognostic factors for disease development
- Correlations between treatment response and genetic predisposition (pharmacogenetics and pharmacogenomics) for personalized medicine to maximize treatment efficiency and avoid adverse drug reactions
- Identification of genetic susceptibility to complex disease and treatment response (ex. inflammatory bowel diseases, asthma and aspirin intolerance, colon adenoma, rheumatoid arthritis, celiac disease, multiple sclerosis, osteoarthritis, uterine leiomyoma, breast cancer, chronic kidney disease etc.)
- Development of biobanks with integrated bioinformatic tools for discovery of genotype/phenotype correlations
- Development of high throughput, reliable and cost effective genotyping including high resolution melting curve analysis
- Development of applications for quantitative gene expression using Real time PCR and for determination of global gene expression profiles using microarrays (biochips)
- Identification of genetic susceptibility to complex diseases and disease clinical features using Single nucleotide polymorphisms (SNP) and haplotype analysis
- Discovery of most efficient genetic and gene expression profiles as disease prognostic and diagnostic biomarkers
- Collaboration with clinical institutions for knowledge transfer into clinical practice for the benefit of the patients
- Genetic analysis and functional cell models

ACHIEVEMENTS

Prof. Uroš Potočnik and Mitja Mitrovič were coauthors of an article published in Nature, which reported 163 gene loci associated with inflammatory bowel diseases (IBD). The study took part in an international consortium of IBD genetics and has identified the so far largest number of associated gene loci for any disease. This is unsurprising considering that the study is one of the largest genome-wide association studies conducted so far with enrolled more than 75000 patients and controls. The Nature paper received more than 1000 citations in just 3 years and is currently most cited paper at University of Maribor.

RESEARCH PROJECTS

INTERNATIONAL COLLABORATION

1. SySPharmPhedia »"Systems pharmacology approach to difficult-to-treat pediatric asthma" call ERA-Net ERACoSysMed "Collaboration on systems medicine funding to promote the implementation of systems biology approaches in clinical research and medical practice, (U. Potočnik coordinator for Slovenian partner, Neatherland, Spain, Germany); 2016-2019;

2. SFRH/BD/79804/2011: The endocannabinoid system in asthma patients and the effect of cannabinoids in the modulation of inflammatory response: supported by Ministry of Science, Portugal, (head of project and mentor: U. Potočnik, PhD student Carina Pinto Kozmus, 2012-2016);

3. BI-US/15-16-061 SLO-USA, (head dr. Uroš Potočnik) "Genetics and pharmacogenomics of chronic immune diseases", collaboration with New York Genome Center, USA (Dr. Tuuli Lappalainen), 2015-2016;

4. International Inflammatory bowel disease Genetics consortium (IIBDGC) (Slovene national consortium coordinator prof. Uroš Potočnik).

NATIONAL PROJECTS

- J3-6785 "Genetics and pharmacogenomics of Inflammatory bowel diseases and genetically related chronic immune diseases" (head dr. Uroš Potočnik), 2014-2017;
- J3-6789 "Pathogenic mechanism of the C9orf72 expanded hexanucleotide repeat mutation in neurodegeneration; 2014-2017;
- IRP-2013: Genetics of self-injury behavior; (Collaboration with University Clinical Center Maribor head UKC MB T. Bunderla; head of project at MF MB U. Potočnik); 2013-2016;
- IRP-2014/01-21: Nucleotide polymorphisms in genes SDF-1α, MMP7, MMP9, TIMP2, RAD18 in MACC1 as prognostic factors for colorectal cancer (head of project at UKC MB M. Horvat; head of project at MF MB U. Potocnik); 2013-2016;
- IRP-2014/01-30: Identificaition of monogenic mutations and DNA polymorphisms associated with risk for Parkinson disease in Slovenian patients (Collaboration with University Clinical Center Maribor head of project at UKC MB D. Flisar, head of project at MF MB U. Potocnik); 2014-2017;
- IRP-2014/01-29: Identification of mutations and DNA polymorphisms in gene SCN9 in patients with complex regional pain syndrome (Collaboration with University Clinical Center Maribor head UKC MB D. Flisar; head of project at MF MB U. Potočnik); 2014-2017;
- IRP-2015: Genetics of chronic kidney disease; (Collaboration with University Clinical Center Maribor head UKC MB S. Bevc; head of project at MF MB U. Potočnik); 2015-2017;
- IRP-2015: Molecular genetic biomarkers for recurrence of Head and neck cancer; (Collaboration with University Clinical Center Maribor head UKC MB A. Čižmarević; head of project at MF MB U. Potočnik); 2015-2018;
- V3-1505 "Analysis and development of rare diseases field in Slovenia", 2015-2017;
- P3-0067 "Pharmacology and pharmacogenomics" (head of project at MF UL Mojca Kržan; prof. U. Potočnik is authorized coordinator of Pharmacogenomics at MF MB, 2014-2017);
- J3-2175 "Genetic risk factors and pharmacogenomics of inflammatory bowel diseases" financed by ARRS (head prof. U. Potočnik, 2009-2012);

• J3-7141-2334 "Genetic risk factors and pharmacogenomics of complex bowel diseases" financed by ARRS (head prof. U. Potočnik, 2005-2008).

SELECTED REFERENCES:

(full bibiliography at http://splet02.izum.si/cobiss/bibliography?code=16340&langbib=eng)

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PHOTODOCUMENTATION OF KEY INSTRUMENTS AND FACILITIES

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Figure 1: Next generation DNA nucleotide sequence analysis system - Illumina MiSeq



Figure 2: Real-time PCR, digital PCR and genotyping system - Life Technologies QuantStudio 12K Flex

LABORATORY FOR CONFOCAL/TWO-PHOTON MICROSCOPY (UPRIGHT) AND CELL ELECTROPHYSIOLOGY:

PL9

GENERAL LABORATORY AND LABORATORY FOR CONFOCAL MICROSCOPY (INVERTED):

1NL4

CORE FACILITY LABORATORY ANIMAL HOUSE:

PL11 – storage

- PL12 general washing room
- PL13 laboratory mouse facility
- PL14 laboratory rat facility
- 3NL1 experimental surgery on rodents

HEAD AND CONTACT PERSONS

Head of Institute:

Assist. Prof. Andraž Stožer, MD PhD

Animal facililty contact: Assist. Maša Skelin Klemen, PhD Prof. Marjan Slak Rupnik, PhD

STAFF

Assist. Maša Skelin Klemen, DVM PhD Assist. Lidija Križančić Bombek, PhD Assist. Prof. Jurij Dolenšek, PhD Prof. Marjan Slak Rupnik, PhD Prof. Gregor Majdič, DVM PhD Prof. Dean Korošak, PhD Assist. Prof. Andraž Stožer, MD PhD Assist. Prof. Marko Gosak, PhD Assist. Prof. Andrej Duh, PhD Assist. Viljem Pohorec, MD Rudi Mlakar, lab manager Ines Kavčič, technical assistant

EQUIPMENT LIST WITH SHORT DESCRIPTIONS

Upright confocal microscope (Leica Microsystems TCS SP5 II)

Top end confocal microscope to acquire prolonged time series of highly spatial and temporal resolved changes in intracellular calcium concentration, membrane potential, and exocytosis in human and animal model tissues using fresh tissue slices.

Inverted confocal microscope (Leica Microsystems TCS SP5 II)

Top end confocal microscope to acquire prolonged time series of highly spatial and temporal resolved changes in intracellular calcium concentration, membrane potential, and exocytosis in human and animal model tissues using fresh tissue slices and cell cultures. In addition, confocal analysis of material surfaces and classical analysis of (patho)histological slides.

System for single cell electrophysiology

A patch-clamp setup to study spontaneous and stimulated electrical activity of excitable cells, ion channels and capacitance based regulated exocytosis using depolarization protocols and slow caged photolysis.

Core facility for laboratory animals

The equipment to house and maintain laboratory mice and rats, both inbred and outbred strains of control, spontaneously mutated as well as transgenic strains to study physiology and pathophysiology of most organ systems.

DESCRIPTION OF LABORATORY ACTIVITIES, REFRENCES AND POSSIBLE LINES OF COLLABORATION

In the last decade we have developed and optimized the fresh pancreas tissue slice method (1) and use it for classical single cell electrophysiology and slow caged compound photolysis (2-6), acquisition of intracellular calcium concentration (7-12), and membrane potential (11) in pancreatic beta cells. Our work continues to yield key knowledge to understand normal physiology of beta cells, the dysfunction of which plays a critical role in pathogenesis of diabetes mellitus. Our methods are applicable also to pancreatic acinar cells and other endocrine tissues (chromaffin cells, pituitary cells) and neurons (13, 14). The most compatible clinical departments are Pathology, Diabetology, and Endocrinology, as well as Abdominal surgery, Ophthalmology and Orthopedics.

PHOTODOCUMENTATION OF KEY INSTRUMENTS AND FACILITIES



Figure 1: Upright confocal microscope and electrophysiology system (multiphoton microscope Leica TCS SP5-II)



Figure 2: Inverted confocal microscope (single-photon inverted microscope Leica TCS SP5-II)



Figure 3: Core facility for laboratory animals

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LABORATORY FOR MOLECULAR PHARMACOLOGY, EXPERIMENTAL TOXICOLOGY AND TRANSLATIONAL MEDICINE – FRLA

HEAD

Assist. Prof. Polonca FERK, M.Pharm. http://sicris.izum.si/search/rsr.aspx?lang=eng&id=15645&opt=1

RESEARCH FELLOWS

- Assist. Katja Jerenec, MD: http://sicris.izum.si/search/rsr.aspx?lang=eng&id=44310&opt=1
- Assist. Marjetka Korpar, PhD, MPharm: http://sicris.izum.si/search/rsr.aspx?lang=eng&id=20231&opt=1
- Assist. Barbara Dariš, PhD, BSc (Biological Sciences): http://sicris.izum.si/search/rsr.aspx?lang=eng&id=20011&opt=1
- Assist. Jan Schmidt, PhD, MPharm: http://sicris.izum.si/search/rsr.aspx?lang=eng&id=43619&opt=1
- Assist. Marko Hojnik, MD
- Assist. Luka Dobovišek, MD
- undergraduate and postgraduate students from the University of Maribor, Faculty of Medicine (voluntary)

LIST AND BRIEF DESCRIPTION OF THE LABORATORY EQUIPMENT

- BioAdvance II (Telstar): laminar flow cabinet for aseptic work
- MCO-19AIC, UV (Sanyo): CO₂ incubator for growing cells
- GBox Chemi XL 1.4 (Syngene): detection system
- Nanodrop 2000c (Thermo Scientific): UV/VIS spectrophotometer for nucleic acid and protein qualification and quantification in 2 μL volume of samples
- LightCycler480, System II (Roche): real-time PCR equipment
- Veriti 96 Well Thermalcycler (Applied Biosystems): thermal cycler for gradient PCR
- Tproffesional Thermocycler (Biometra): thermal cycler for gradient PCR
- Infinite M200Pro (Tecan): microtiter plate reader
- DM6000 B+ BDFC365 FX (Leica): inverted research microscope with fluorescence detection camera
- iBlot (Invitrogen, Life Technologies): quick dry blotting system
- BenchPro 4100 (Invitrogen, Life Technologies): card processing station western blot autostainer
- other equipment
 - o autoclave A-65 V (Kambič)
 - Forma 900 Series (Thermo Scientific): laboratory freezer -86 °C
 - laboratory refrigerator +4 °C do +8 °C (Kirsch)
 - laboratory freezer -30 °C (Kirsch)
 - o liquid nitrogen container (Thermo Scientific)
 - o portable container with liquid nitrogen (Thermo Scientific)
 - o centrifuge 5430 R (Eppendorf)
 - centrifuge LMC-3000 (Biosan)
 - Thermomixer comfort (Eppendorf)
 - Vibromix (Tehtnica)
 - Minispin centrifuges (Starlab)

o gel electrophoresis equipment (Invitrogen)

DESCRIPTION OF THE LABORATORY ACTIVITIES AND POSSIBILITIES FOR COOPERATION

Mission

- integration of preclinical basic knowledge with its usefulness in clinical practice, translational medicine
- improving effectiveness and safety of pharmacological treatment
- optimization, rationalization and individualization of polypharmacotherapy, personalized medicine
- awareness of the importance of applied clinical pharmacy and clinical pharmacology
- interdisciplinary work

Research fields

- 1. Molecular pharmacology
 - growing of various primary cell cultures and cell lines
 - testing: cell viability, metabolic activity, proliferation, cytotoxicity, apoptotic pathways, cell differentiation, biocompatibility
 - preclinical *in vitro* testing of pharmacodynamic properties of new potential active substances, (dose) concentration response relationship
 - exploring molecular mechanisms of action of active substances, studying signaling pathways (detection of DNA polymorphisms, expression of RNA, miRNA, siRNA, proteins), identifying new drug target molecules
 - exploring mutual interactions of active substances at the molecular level, intermolecular interactions; interpretation of additive, synergistic & antagonistic interactions
 - evaluation of interactions between cannabinoids and oestrogens in primary human osteoblasts
 - evaluation of interactions between cannabinoids and oestrogens in melanoma cells
 - evaluation of the effects of UV-filters and potential herbal new anti-cancer agents on melanoma cells
 - evaluation of the effects of extracts of rosemary on melanoma cells
 - biocompatibility testing of selected potentially clinically useful polymers, prepared using green technologies, in primary human osteoblasts
- 1. Pharmacogenetics/pharmacogenomics, human genetics & personalised medicine in multifactorial diseases
 - analysis of genetic, epigenetic and other (clinical, biochemical, environmental, ...) prognostic factors and biomarkers for the disease prognosis and for monitoring pharmacological treatment efficacy and safety in patients with polycystic ovary syndrome, with type 2 diabetes (endocrinopharmacology), in pediatric patients with persistent asthma or. with allergic rhinitis, patients with breast cancer, patients after liver transplantation
 - evaluation of (pharmaco)genomic results and development of relevant prognostic biostatistical models (in collaboration)

- identification of genetic, epigenetic and other risk factors for male and female infertility, polycystic ovary syndrome, premature ovarian failure, ovarian hyperstimulation syndrome, breast cancer
- isolation of DNA, RNA and proteins from different types of biological materials, (realtime) PCR, determination of genetic polymorphisms, DNA sequencing (NGS), western blot
- Pharmacoepidemiology
 - analysis of trends in outpatient prescribing of drugs and drug consumption in Slovenia
 - checking the adequacy of outpatient co-prescribed medication in order to ensure efficient and safe polypharmacotherapy
 - analysis of suitability of prescribed drug combinations in conjunction with data obtained under the project Quality of HealthCare in Slovenia
- Interventional studies with dietary supplements, clinical studies, translational medicine
 - planning and implementation of intervention studies with food supplements (in cooperation)
 - $\circ\,$ support in planning and implementing post-marketing clinical studies (in collaboration)
 - \circ development of web-based clinical information system for standardized data collection
 - \circ providing effective and secure IT support
 - \circ cooperation in biostatistical analyses
 - \circ interpreting the results from the pharmacological point of view
- Toxicology
 - cell culture studies of toxic effects of different pharmacologically active compounds, environmental pollutants and endocrine disrupting compounds, used in food industry
 - *in silico* methods for toxicity prediction
 - phase 1 and phase 2 metabolism of xenobiotics
 - regulatory toxicology
 - identification and determination of toxicological compounds
- Other
 - modeling of pharmacological and toxicological parameters in simulation environment, simulation scenarios
 - developing virtual patient cases with the integration of pharmacological knowledge and basic knowledge of clinical and research information systems
 - telepharmacology
 - academic doping

Current research collaborations

- University of Maribor, Faculty of Chemistry and Chemical Engineering
- University Medical Centre Maribor
- University of Ljubljana, Faculty of Pharmacy
- Active collaboration in ELIXIR node Slovenia: <u>https://www.elixir-europe.org/about/elixir-slovenia</u>

- University of Ljubljana, Faculty of Medicine: Institute for Pharmacology & Experimental Toxicology, Institute for Biostatistics & Medical Informatics, Department of Obstetrics & Gynaecology
- National Institute of Chemistry, Ljubljana
- University Medical Centre Ljubljana
- University of Belgrade, Faculty of Medicine, Institute for Pharmacology, Clinical Pharmacology & Toxicology
- Ars Pharmae d.o.o., Slovenia

LABORATORY EQUIPMENT - PHOTOS



Figure 1: Laminar flow cabinet in FRLA (own photo)



Figure 2: CO_2 incubator for growing cells, liquid nitrogen container, portable container with liquid nitrogen, all in FRLA (own photo)



Figure 3: Inverted research microscope in FRLA (own photo)



Figure 4: UV/VIS spectrophotometer for using 2 µL volume of samples, in FRLA (own photo)



Figure 5: Microtiter plate reader in FRLA (own photo)



Figure 6: Real-time PCR equipment in FRLA (own photo)



Figure 7: Thermal cyclers for gradient PCR in FRLA (own photo)



Figure 8: Card Processing Station Western Blot Autostainer in FRLA (own photo)

INSTITUTE OF BIOMEDICAL SCIENCES

HEAD

• Assist. prof. dr. Uroš Maver (<u>uros.maver@um.si</u>)

TEAM

- Assist. dr. Janja Stergar, postdoc
- Lidija Gradišnik, researcher
- Eneko Madorran, researcher
- Boštjan Krajnc, technician

IMPORTANT EQUIPMENT WITH SHORT DESCRIPTION

Equipment	Type and producer	Short description
Automatic Franz Diffusion Cell System	Logan Sytem 912-6, Logan Instruments Corp., USA	A standard system designed to simulate transdermal and topical administration routes, as well application on open wounds.
		It enables use in applications, where it is necessary to test in smaller volumes (5 to 15 mL)
Infrared spectroscopy	Cary 630 FTIR, Agilent Technologies, USA	A system for evaluating the spectroscopic properties of matter, visible in the IR field (identification of the substance and real-time follow-up of structural changes)
UV/VIS spectrophotometry	Cary 60 UV/VIS, Agilent Technologies, USA	A system for determination of the concentration of substances in solution, as well as the kinetics of processes taking place in the liquid state
Digital hydraulic press	CrushIR, PIKE Technologies, UK	Laboratory press for production of low batches of tablets
Spin-coating system	SPIN-150i-NPP, S.P.S. Vertriebs GmbH, Germany	Modern spin coater for precise thin film preparation, useful especially for preparation of all kinds of coatings (even multi-layer) for various applications, e.g. model wound dressings.
Inverted optical microscope	Axiovert 40, Zeiss, Germany	Inverted optical microscope for cell culture applications.
Microplate reader	Varioskan, ThermoScientific	Fast determination of fluorescence, luminiscence etc. of a large number of samples.
Cell analyzer	MUSE, EMD Millipore, USA	Cell counter and analyser with a versatile applicability options. In can be used to develop own methods or use commercial kits to test for different cell related phenomena (e.g. phagocytosis, apoptosis,)

Biochemical analysis	Cobas c 111, Roche, Switzerland	Compact solution for clinical chemistry testing in laboratories running up to 50 samples per day or a throughput up to 25,000 tests per year.
HPLC system	Waters 1525, Waters Corp., USA	Separation, identification and quantification of components in solution.
Imaging flow cytometry	Amnis Imagestream X mkII, EMD Millipore, USA	Imaging flow cytometry, which combines regular flow cytometry with multi-channel cell visualization.

LABORATORY FOCUS

- 1. Development of in vitro testing methods based on human derived cell cutlures:
 - Cell isolation from human tissues
 - o Full cell characterization for test standardization
 - Development of novel *in vitro* models for material testing in terms of safety and pharmacotherapeutic efficiency (monolayers, co-cultures, in the future also 3D models)
 - Biocompatibility and cytotoxicity assessment (based on different cell lines and standard, as well newly developed assays)
- 2. Biomedical applications:
 - Development of drug delivery systems (focus especially skin pharmacology either wounds and wound treatment or skin cancer)
 - Development of novel solutions in wound healing applications (chronic and acute wounds)
 - Tissue engineering applications (new solution in relation to various tissues, ranging from soft e.g. skin brain/spine, intestines..., to hard bone...)
- 3. In vitro toxicology
 - Development of novel *in vitro* models for testing of potential toxic effects of various substances (ranging from natural, to waste materials, as well as drugs)
 - o The focus is on physiological studies, rather than just defining potential toxicity
 - The goal is to achieve a model that could close the gap between preclinical and clinical studies.

CURRENTLY RUNNING PROJECTS

National level

1. Bio-responsive magneto-optically coupled nanomaterial-based systems for innovative skin cancer treatments

Project page: Link to the project

Short description: This project addresses new concepts in skin cancer treatment. The proposed smart diagnostic, targeted drug delivery and stimuli-responsive release system is based on hybrid optically and magnetically active nanoparticles, which facilitate both, focused localization and extraction of hybrid nanoparticles by using an external magnetic field, and photothermally-responsive drug release and treatment.

2. Genetics and pharmacogenomics of inflammatory bowel diseases and genetically related chronic immune diseases

Project page: Link to the project

Short description: The goal of this project is to improve eQTL analysis with measurement of gene expression in leukocytes, obtained from biopsy of intestinal tissues from patients suffering from inflammatory bowel disease.

3. Multifunctional electrospunned nanofibers development and dynamic interaction studies with pathogen bacteria

Project page: Link to the project

Short description: This project address the development of advanced multifunctional electrospunned nanofibers, manufactured from biodegradable polymers and natural extracts, that would provide structural and chemical support for wound repair with simultaneous antimicrobial and antioxidant functions and will offer great pharmaceutical potentials as bioresorbable antimicrobial material for enhanced wound care.

4. Electrostatic immobilisation of bacterial cells and effects on their physiology

Project page: <u>Link to the project</u>

Short description: The aims in this project are to: (i) develop an LBL immobilization strategy for bacterial cells, (ii) characterize physicochemically and microscopically such LBL immobilized bacteria, (iii) determine the effect of polyelectrolyte encapsulation on physiology and cell division and (iv) to evaluate the changes of mass balances of LBL immobilized cells in comparison to free-living cells.

European level

1. AEROWOOD

Project page: <u>http://blogs.helsinki.fi/aerowood-project/</u>

Short description: development and study of novel porous and lightweight aerogels, based on isolated raw resources from wood components, for biomedical applications.

2. BIOSHAPES

Project page: <u>http://www.bioshapes.net/</u>

Short description: development and preparation of novel materials and formulations thereof from polysaccharides for use in different value chains (health, food industry, packaging etc.)

Clinical (in collaboration with University Medical Centre Maribor)

- 1. Vitamin D deficiency among pregnant women and newborns (together with <u>Division of Gynecology</u> <u>and Perinatology</u>).
- 2. New approaches to transdermal treatment of nodular basal cell carcinoma through controlled delivery of imiquimod together with <u>Department of Dermatology and Venereal Diseases</u>).
- 3. The impact of electromagnetic waves on the transformation of astrocytes (together with <u>Department of Neurosurgery</u>).
- 4. Influence of local delivered insulin on healing of superficial dermal wounds (together with <u>Department of Plastic and Reconstructive Surgery</u>).

- 5. Preparation of advanced medical materials with incorporated analgesic active ingredients for less painful healing of superficial dermal wounds (together with <u>Department of Plastic and</u> <u>Reconstructive Surgery</u>).
- 6. Pharmacotherapeutic contact lenses for innovative approach in treating eye diseases (together with <u>Department of Ophthalmology</u>).

PhD theses

At the moment we overview five PhD candidates (two almost finished, two only started), whose theses are related to the main topics of the Institute of Biomedical Sciences (wounds, tissue engineering and skin pharmacology).

IMPORTANT PUBLICATIONS (2015-2016)

- FINŠGAR, Matjaž, PERVA-UZUNALIĆ, Amra, STERGAR, Janja, GRADIŠNIK, Lidija, MAVER, Uroš. Novel chitosan/diclofenac coatings on medical grade stainless steel for hip replacement applications. *Scientific reports*, ISSN 2045-2322, Published online:24 May 2016, vol. 6, art. no. 26653, str. 1-17, doi: 10.1038/srep26653.
- NARANĐA, Jakob, SUŠEC, Maja, MAVER, Uroš, GRADIŠNIK, Lidija, GORENJAK, Mario, VUKASOVIĆ, Andreja, IVKOVIĆ, Alan, RUPNIK, Marjan, VOGRIN, Matjaž, KRAJNC, Peter. Polyester type polyHIPE scaffolds with an interconnected porous structure for cartilage regeneration. *Scientific reports*, ISSN 2045-2322, Published online: 24 June 2016, vol. 6, art. no. 28695, str. 1-11, doi: 10.1038/srep28695.
- EHMANN, Heike M. A., BREITWIESER, Doris, WINTER, Sascha, GSPAN, Christian, KORAIMANN, Günther, MAVER, Uroš, ŠEGA, Marija, KÖSTLER, Stefan, STANA-KLEINSCHEK, Karin, SPIRK, Stefan, RIBITSCH, Volker. Gold nanoparticles in the engineering of antibacterial and anticoagulant surfaces. *Carbohydrate polymers*, ISSN 0144-8617. [Print ed.], 2015, vol. 117, str. 34-42, ilustr., doi: 10.1016/j.carbpol.2014.08.116.
- MAVER, Tina, MAVER, Uroš, MOSTEGEL, Florian, GRIEßER, Thomas, SPIRK, Stefan, SMRKE, Dragica, STANA-KLEINSCHEK, Karin. Cellulose based thin films as a platform for drug release studies to mimick wound dressing materials. *Cellulose*, ISSN 0969-0239, Feb. 2015, vol. 22, iss. 1, str. 749-761, ilustr., doi: 10.1007/s10570-014-0515-9.
- MAVER, Tina, HRIBERNIK, Silvo, MOHAN, Tamilselvan, SMRKE, Dragica, MAVER, Uroš, STANA-KLEINSCHEK, Karin. Functional wound dressing materials with highly tunable drug release properties. *RSC advances*, ISSN 2046-2069, 2015, vol. 5, iss. 95, str. 77873-77884. http://pubs.rsc.org/en/content/articlepdf/2015/ra/c5ra11972c, doi: 10.1039/C5RA11972C.
- NADRAH, Peter, MAVER, Uroš, JEMEC, Anita, TIŠLER, Tatjana, BELE, Marjan, DRAŽIĆ, Goran, BENČINA, Mojca, PINTAR, Albin, PLANINŠEK, Odon, GABERŠČEK, Miran. Hindered disulfide bonds to regulate release rate of model drug from mesoporous silica. ACS applied materials & interfaces, ISSN 1944-8244. [Print ed.], 2013, vol. 5, issue 9, str. 3908-3915, doi: 10.1021/am400604d.
- UKMAR GODEC, Tina, MAVER, Uroš, PLANINŠEK, Odon, KAUČIČ, Venčeslav, GABERŠČEK, Miran, GODEC, Aljaž. Understanding controlled drug release from mesoporous silicates : theory and experiment. *Journal of controlled release*, ISSN 0168-3659. [Print ed.], 2011, vol. 155, issue 3, str. 409-417, ilustr.
- STERGAR, Janja, MAVER, Uroš. Review of aerogel-based materials in biomedical applications. *Journal of sol-gel science and technology*, ISSN 1573-4846, 2016, vol. 77, iss. 3, str. 738-752.

- MAVER, Uroš, VELNAR, Tomaž, GABERŠČEK, Miran, PLANINŠEK, Odon, FINŠGAR, Matjaž. Recent progressive use of atomic force microscopy in biomedical applications. *TrAC, Trends in analytical chemistry*, ISSN 0165-9936, Jun. 2016, vol. 80, str. 96-111, doi: 10.1016/j.trac.2016.03.014.
- MAVER, Tina, MAVER, Uroš, STANA-KLEINSCHEK, Karin, SMRKE, Dragica, KREFT, Samo. A review of herbal medicines in wound healing. *International journal of dermatology*, ISSN 0011-9059. [Print ed.], Jul. 2015, vol. 54, iss. 7, str. 740-751. http://onlinelibrary.wiley.com/doi/10.1111/ijd.12766/abstract, doi: 10.1111/ijd.12766.

LABORATORY 1NL5		
Photo	Equipment	
	Automatic Franz Diffusion Cell System	
	Infrared spectroscopy	
	UV/VIS spectrophotometry	

EQUIPMENT IN PHOTOGRAPHS

	Digital hydraulic press
	Spin-coating system
LABORA	IORY PL3
Photo	Equipment
	Microplate reader
	Inverted optical microscope



LABORATORY FOR MICROBIOLOGY

HEAD

prof. dr. Maja Rupnik

RESEARCHERS dr. Sandra Janežič

Sabina Žalig

Tanja Rikanović

Aleksander Kocuvan

THE LIST OF AVAILABLE EQUIPMENT AND METHODS

The equipment and methods available in Laboratory for microbiology include

- aerobic and anaerobic cultivation techniques (anaerobe workstation A35);
- preparation of biological samples (sonication; ultracentrifugation up to 5 ml at 150.000 rpm on table top ultracentrifuge Optima MAX-XP);
- molecular biology (preparation and analysis of nucleic acids, different amplification approaches);
- sequencing of bacterial and phage genomes and complex microbial populations (MiSeq platform).

RESEARCH EXPERTISE AND COLLABORATION POSSIBILITIES

Research is focused at two main topics: pathobiology and molecular epidemiology of bacterium *Clostridium difficile* and the role of gut microbiota in health and disease.

Additional expertise on detection, identification and characterization of different microorganisms, molecular typing methods, host-pathogen interactions and characterization of complex microbial communities.

Collaborative projects include detection of rare and unusual microorganisms, interaction of microorganisms with different biomedical materials, comparative studies of microbial communities in different patient groups (gut microbiota in inflammatory bowel disease; microbiota of chronic wounds) and comparative genomics of bacterial pathogens including detection of virulence and resistance genes.





PHOTODOCUMENTATION OF KEY INSTRUMENTS AND FACILITIES



LABORATORY OF CELL BIOLOGY, N2L8

THE HEAD OF THE LABORATORY Assoc. prof. dr. Saška Lipovšek

CO-WORKERS Assist. Dr. Barbara Dariš tehnical assist. Marijana Knez

THE LIST OF THE EQUIPMENT

- Stereomicroscope Nikon SMZ1000: allows up to 8x magnifications of the specimens.
- Microskope Nikon ECLIPSE Ci: allows 40x, 100x, 200x, 400x in 1000x magnifications of the structures.
- Knifemaker LEICA EM KMR3: enables the production of the glass knives for cutting semi-thin and ultra-thin tissue sections.
- Specimen trimming device LEICA EM TRIM2: for trimming of biological samples prior microtomy.
- Microtome LEICA RM 2265: for cutting semi-thin and ulta-thin sections of the tissue embedded into epoxy resins.
- Stretching table Medite OTS 40: for stretching, drying and staining of the tissue sections.

ACTIVITIES OF THE LABORATORY

In the laboratory of cell biology tissue samples are prepared. Tissue preparation for microscopy includes many steps. Common procedures include fixation of the material, embedding of the samples into epoxy resins, sectioning to produce thin, translucent tissue slices and staining of the tissue slices. Tissue samples are prepared using specimen trimming device LEICA EM TRIM2. Tissue slices are cut using glass knife mounted in the microtome. At the end tissue slices are dryed and stained using stretching table Medite OTS 40. The goal of these methods is tissue analysis by light microscopy and transmission electron microscopy.

FIGURES OF THE EQUIPMENT

Stereomicroscope Nikon SMZ1000



Microscope Nikon ECLIPSE Ci



Knifemaker LEICA EM KMR3



Specimen trimming device LEICA EM TRIM2



Microtome LEICA RM 2265



Stretching table Medite OTS 40



LABORATORY OF BIOPHYSICS

HEAD OF LABORATORY Full Professor Dr. Milan Brumen, STAFF

Full Professor Dr. Milan Brumen, head of laboratory Assistant Professor Dr. Andrej Dobovišek, teaching and research assistant Petra Rogan, MSc. in Medical Physics, teaching and research assistant Aleksander Kocuvan, BSs in Physics and Biology, professional co-worker

PEDAGOGICAL WORK

Laboratory of Biophysics possesses many of basic as well as high-tech didactic experimental equipment like: computer supported ultrasound measuring system, diagnostic ultrasound apparatus, computer supported X- ray measuring system, infra-red camera, computer supported didactic measuring system for biomechanics of motion, optical spectrometry, didactic membrane potential measuring system, many other basic physical experiments supporting studies in medical physics.

RESEARCH WORK

As researchers we do basic as well as applied research in the field of theoretical biophysics by using methods of mathematical physical simulations. We are involved in the following research problems: biophysics of red blood cells, biophysics of the cell membrane, mathematical physical simulations of intra and inter-cellular calcium oscillations and signalization pathways in airway smooth muscle contraction, mathematical physical simulations of eicosanoids production in inflammatory cells and simulations of the impact of NSAIDs on aspirin intolerance in asthmatic patients, research of maximum entropy production principle (MEPP) in enzyme reactions, modelling of colloidal systems and research of physical properties of polymeric membranes.

The results of our research work are published in international scientific journals as original and review papers as well as chapters in scientific monographs published by internationally established publishers. We collaborate with many foreign as well as domestic research institutions and scientists.

PHOTODOCUMENTATION OF KEY INSTRUMENTS AND FACILITIES



Geometric optics experiment



Computer supported merasurement by ultrasound measuring system

LABORATORY OF CHEMISTRY

HEAD of laboratory Full professor Željko KNEZ, Ph.D.

RESEARCHERS

Assist. Petra KOTNIK, Ph.D.

Staša JURGEC

LIST OF APPARATUS AND BRIEF DESCRIPTION:

- Liquid chromatography mass spectrometry (LC-MS/MS) Agilent 1200 HPLC + Agilent 6460 MSD
- Liquid chromatography mass spectrometry enables qualitative and quantitative analysis
 of analytes and their metabolites by detecting molar weight of ions and their fragments.
 LC-MS/MS triple quadrupole (QQQ) with JetStream ionisation offers highly sensitive
 detection in molecule range between 15 3000 Da. The main application of LC-MS/MS is
 quantification of active compounds and their metabolites from "in vitro" and "in vivo"
 samples.
- Applications:
 - Clinical and preclinical studies;
 - Therapeutic monitoring of drugs;
 - Identification of metabolites, impurities...
 - Detection of pesticides in water, food products...
 - Detection of macromolecules in medicine and pharmacy proteins, amino acids, biomarkers, monoclonal antibodies (known compounds);
 - Detection of mononucleotides, vitamins, mycotoxins, metabolites, fatty acids, sterols, etc. in biological matrices;
 - Forensic applications the presence of prohibited substances in biological matrices;
 - Toxicology quantification of drugs and their half-products, metabolites in biological and non-biological media.
- Headspace gas chromatography with mass spectrometry (HS-GC-MS) Shimadzu GCMS – QP2010 Ultra
- GC-MS is used in forensics, analyses of pesticides and food safety, pharmacy and clinical toxicology, medicine and in analyses of food and fragrances. The method provides rapid qualitative and quantitative analysis of volatile and less volatile organic compounds from complex samples; determination of molecular weight and composition of unknown organic compounds from complex biological and non-biological samples. Identification of sample components is enabled using mass spectra database (spectrum library).
- High performance thin layer chromatography (HPTLC) Camag
- High performance thin layer chromatography (HPTLC) is an analytical method where separation of components is performed on a chromatographic plate. The separation is enabled due to differences in distribution coefficients of the analyte. HPTLC method is

used for determination of purity and identification of compounds, for verification of the performance after isolation and purification of compounds and for separation of mixtures which cannot be separated using other methods such as crystallization, distillation, sublimation and others.

- Total Organic Carbon analyser TOC Shimadzu
- TOC »Total Organic Carbon« analyser allows indirect measurement of total carbon from organic molecules dissolved in liquids. Sensitivity is determined by detecting carbon after catalytic oxidation. For determination of total carbon (TC), inorganic carbon (IC), total organic carbon (TOC) and non-purgeable organic carbon (NPOC), different measuring methods can be used depending on the sample. TOC is used for determination of organic molecules in drinking water, waste and industrial waters, pharmaceutical waters, in water reaction media, etc.
- Dissolution test after USP in EP standardization methods Agilent 708-DS
 The apparatus is designed for the release of pharmaceutical substances (in different
 pharmaceutical forms) in different media under USP and EP standard methods. It allows
 carrying out eight experiments simultaneously with manual sampling. Using this
 apparatus, the dissolution rate of the substance and change of enthalpy at dissolution can
 be determined.
- Rotational viscometer Anton Paar Rheolab QC
- Rotational viscometer Rheolab QC is an apparatus for measuring shear stress, shear rate, speed, viscosity at different temperatures, and for observing physical changes of materials, such as softening, melting, solidification, crystallization and elasticity. The apparatus is useful for determination of rheological properties of highly viscous samples (oils, polymers...)

DESCRIPTION OF ACTIVITIES OF THE LABORATORY AND THE POSSIBILITY OF COOPERATION

In the Laboratory for Chemistry at the Faculty of Medicine at UM the teaching and research activities are actively implemented. In the context of teaching activities, lectures and laboratory exercises in Chemistry for undergraduate study program "General Medicine" and "Membrane mass transport phenomena" for <u>postgraduate study program "Biomedical Technology"</u> are performed.

Basic research efforts focus on separation processes, particularly the development of new separation and analytical methods and studies of antioxidativity and stability of pharmaceutical and natural ingredients. The research is focused on proteomics, especially development of analytical methods using LC-MS/MS for determination of large molecules, such as proteins and peptides. Techniques such as liquid chromatography (LC), gas chromatography (GC), thin layer chromatography (TLC), mass spectrometry (MS), dissolution test of pharmaceutical substances under USP and EP standard methods, techniques of rheology (rotational viscometer) and determination of total organic carbon in liquids (TOC) have been successfully introduced.

The laboratory successfully cooperates with the Laboratory of Separation Processes and Product Design at the Faculty of Chemistry and Chemical Engineering at UM, with other laboratories at the Faculty of Medicine (UM) and with the University Medical Centre Maribor in several projects within the Public Research Agency of the Republic of Slovenia and in the program group P2-0046. Participation includes basic and applied research and service activities. We see the opportunity for cooperation in the fields of chemistry, biochemistry, pharmacology, toxicology, proteomics, forensics, medical diagnostics, etc.

PICTURES OF APPARATUS



Liquid chromatography - mass spectrometry (LC-MS/MS) – Agilent 1200 + 6460 JetStream



Headspace - gas chromatography - mass spectrometry (HS-GC-MS) – Shimadzu QP2010 Ultra



High performance thin layer chromatography (HPTLC) - Camag



Total Organic Carbon analyser - TOC Shimadzu



Rotational viscometer - Rheolab QC Anton Paar



Dissolution test after USP in EP standardization methods – Agilent 708 DS

LABORATORY FOR MACROSCOPIC AND SURGICAL ANATOMY

Institute for anatomy, Ljubljanska 5

HEAD Full Prof. dr. Božena Pejković

STAFF

Asist. dr. Lidija Kocbek Asist dr. Mateja Zemljič Miroslav Karlo, tehn. sod.

PHOTOS OF EQUIPMENT WITH SHORT SURVEY



Dissection table for preparing the cadaver for embalming procedure with the tank for the cadavers



Refrigerators and injection system for the cadavers



Dissection and exercise room for macroscopic anatomy

In this laboratory the cadavers are embalmed using embalming procedure after prof. Thiel and prof. Anderhuber. When the embalming is completed, the cadavers are embedded into the tank for six months untill approximately one year to be prepared for anatomic dissection. Beside the dissecting courses for the medical students, in this laboratory are organized hands on workshops for the surgeons of all surgical specialities.

LABORATORY FOR THE FUNCTIONAL AND CLINICAL ANATOMY (3NL2)

HEAD

Full Prof. dr. Božena Pejković

STAFF Asist. dr. Lidija Kocbek Asist dr. Mateja Zemljič Asist. Sašo Pjević Asist. Iztok Caglič

In this laboratory there is no permanently installed equipment. When necessary we bring it from elsewhere. In this laboratory we do the research in functional and clinical anatomy.

LABORATORY FOR VIRTUAL ANATOMY (P26)



In this laboratory the students do the exercises in virtual anatomy on virtual anatomic specimens and excercises in clinical anatomy on virtual clinical cases, that are based on real persons.

LABORATORY FOR FUNCTIONAL AND COMPARATIVE ANATOMY (3NL4)

HEAD Full Prof. dr. Božena Pejković

STAFF

Asist. dr. Lidija Kocbek

Asist dr. Mateja Zemljič

Asist. dr. Miha Munda

Asist. Sašo Pjević

Asist. Iztok Caglič

In this laboratory there is no permanently installed equipment. When necessary we bring it from elsewhere. In this laboratory we do the research in functional and comparative anatomy.

LABORATORY FOR MICROSCOPIC ANATOMY AND HISTOLOGY(3NL6)

HEAD Full Prof. dr. Božena Pejković

STAFF Asist. dr. Miha Munda Asist. dr. Lidija Kocbek Asist dr. Mateja Zemljič Andreja Robič, tehn. sod.

LIST OF EQUIPMENT WITH SHORT SURVEY

Tissue processor, microtome, paraffin embedding station, stainers, diagnostic microscope with camera, 3D stereo magnifyer - microscope for 3D object observation with camera. In this laboratory we do the research in microscopic anatomy and histology: microanatomic and histologic preparations, the research in imunohistochemistry, analysis of photos.



Tissue processor



Paraffin embedding station with thermostat



Paraffin embedding station



Water bath, microtome and cooling plate



Automatic stainer



Automatic stainer for imunohistochemical research



Diagnostic microscope with camera



3D stereo magnifyer - microscope for 3D object observation with camera