Preface

Complex illnesses like cancer, cardiovascular diseases, multiple sclerosis, Alzheimer’s and Parkinson’s disease as well as different kinds of serious inflammatory or infectious diseases (e.g. HIV) have a tremendous negative impact, not only on the patient himself but also on the whole society and linked social and insurance systems. These diseases have been key topics in the broad field of biomedical research in the past decades on a global level, and large amounts of investigations and studies have been carried out. However, appropriate diagnosis, treatments as well as preventive methods still pose one of the biggest challenges that society faces.

Advanced nano- and biomaterials for medical applications that facilitate new methods and multidisciplinary approaches promise to tackle these challenges.

As one of the most promising KETs, nanotechnology facilitates major breakthroughs in different application sectors. Nanomedicine, the application of nanotechnology to health, raises high expectations for millions of patients for better, more efficient and affordable healthcare, and promises new solutions to improve medical treatments. Moreover, fundamental research in nanomedicine allows a better understanding of biological processes in the human body at molecular and nanometric level. Several areas of medical care are already benefiting from the advantages that nanotechnology can offer. The present nanomedicine folder compiles expertise of different research groups in the field of

(i) Diagnostics & Imaging,
(ii) Therapeutics and
(iii) Regenerative Medicine.

We would highly appreciate to expand the portfolio with your expertise. If you are interested in further information and how to become involved, please contact: office@nanomedicine-austria.at.
## Part I - DIAGNOSTICS & IMAGING

- Fluorescence Microscopy below the Diffraction Limit
- LabDisk: a Multi-Purpose, Multi-Target Diagnostic Tool for Patient Management at the Point-of-Care
- Metabolomics for Nanomedicine
- Multi-Omics Fusion Technology and Targeted Molecular Imaging: a Fully Integrated Approach for Biomarker Research in Precision Medicine
- Nanoprobe-Based Mix & Measure Immunodiagnostcs
- Targeted Nanoparticles for Medical Imaging of Atherosclerotic Plaques

## Part II - THERAPEUTICS

- Advanced Liposome Technology for Targeted Drug Delivery
- Continuous Manufacturing of Solid Nano-Formulations
- Immunological Characterization of Novel Nanoparticulate Formulations
- Investigations on the Tumor-Biology in Aggressive B Cell Lymphomas - Development of Anti-Cancer Therapies for Aggressive B Cell Lymphoma
- Magnetic Nanoparticles for Biomedical Applications: Modeling and Monitoring of In Vivo Distribution
- Modeling of Biological Barriers to Study Therapeutic Relevant Nanoparticle Interactions
- Open Flow Microperfusion
- PlaZentaTox: A Human Ex-Vivo Placental Transfer Model
- Surface Modification of PLGA Micro- and Nanoparticles
- Therapy of Bladder Diseases

## Part III - REGENERATIVE MEDICINE

- Macro-Molecular Crowding (MMC): A New Approach to Mimic Vocal Fold Scar Under In-Vitro Conditions
- Skin Wound Healing – Skin Regeneration, Repair and Reconstruction
- Impressum
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Fluorescence Microscopy below the Diffraction Limit

In 2006, three seminal papers appeared simultaneously, demonstrating the feasibility of optical microscopy below the diffraction limit based on single molecule observations (1-3). Since then, it has become a widely accepted tool for high resolution investigations in cell biology, and is well known under the acronyms PALM or STORM.

The principle is conceptually simple: it makes use of the phenomenon that fluorescent dye molecules can be stochastically switched between dark and bright states. The idea is sketched in the figure: assume a structure with a size of a few hundred nanometers. If all dyes were fluorescing simultaneously, the individual point spread functions would overlap due to their proximity to one another, concealing the details of the structure (a). If only a few dye molecules are in the bright state at the same time (b), so that the signals do not overlap anymore, the individual locations of the molecules can be calculated very accurately.

If such images are recorded repetitively, other dye molecules will become visible in the consecutive images; one can thereby determine the positions of all dye molecules and merge them into a super-resolution image that now reflects the details of the original structure. An example can be seen in the right column: the membrane of neurons was fluorescently labelled and imaged by means of a blinking GFP mutant. Panel a shows a section of how it appears using standard fluorescence microscopy; b shows the super-resolution version.

The Biophysics group at TU Wien uses super-resolution microscopy in various cell biological contexts at a routine basis. Examples include the differential analysis of the subcellular localization of mitochondrial proteins (4), or of signaling molecules in the plasma membrane of T cells (5). The latter study revealed the importance of a thorough spectroscopic knowledge for correct data interpretation. It turned out that although subdiffraction structures can be nicely imaged, these images are not free of artifacts. Overcounting of dye positions can easily give rise to apparent clusters, which need to be discriminated from real protein clusters. A methodological framework has been put forward and is now being continuously refined to provide a robust basis for studying cell biological structures at a resolution of about 20nm.

The principle of super-resolution microscopy using blinking dye molecules. (a) shows standard diffraction limited fluorescence microscopy images, (b) shows superresolution microscopy images. From left to right: a sketch showing the locations of the dye molecules, simulated image of the structure, real data of a neuron labeled with a plasma membrane-localized fluorophore.
**LabDisk: a Multi-Purpose, Multi-Target Diagnostic Tool for Patient Management at the Point-of-Care**

The LabDisk is a CD-shaped plastic disposable chip that performs fully automated molecular analysis by microfluidically integrating analytical steps that would otherwise need an entire laboratory and many instruments to be performed—e.g. extraction, purification, amplification, and detection of DNA/RNA.

After inserting the sample, the pathogens are lysed, DNA/RNA is extracted and purified using an on-disk pre-stored bead- and buffer-based extraction kit. The purified DNA/RNA is mixed with lyophilized amplification reagents and then distributed into multiplexed reaction chambers where it rehydrates the pre-stored primers/probes. The amplification starts (either qPCR or isothermal) and is detected in real time via fluorescence. A compact and portable processing device is used for disk handling, integrating heating and detection units. The LabDisk is a generic platform that is usable in applications ranging from healthcare to veterinary, environmental, and food analysis.

The main innovative features of the LabDisk are:

1. Modular nature (based on microfluidic unit operations that can be adapted and interfaced easily and rapidly).
2. Adaptability to disease panels, according to local needs (endemic, epidemic).
3. High degree of interoperability: nucleic acid, protein, clinical chemistry on the same platform and device.
4. The manufacturing is based on microthermoforming of polymer foils, and injection molding, allowing the platform to be available in small/middle-scale (for clinical validation) and large scale (for product development).

**Reference projects**
- FP7 DiscoGnosis
- H2020 DIAGORAS
- H2020 DMC-MALVEC

**Reference video**
- "Lab-on-a-disk to diagnose Malaria" (FP7 DiscoGnosis)
Metabolomics for Nanomedicine

Metabolomics is the latest “omics”-branch which has a focus on the identification and quantification of metabolites, small molecules (<1500 Da) that provide a highly integrated measure by comprehensively reflecting the effect of genetic, proteomic and environmental changes. Metabolic profiling investigates changes in the concentrations of endogenous metabolites in tissues or body fluids following nanomedical interventions. Interventions can lead to perturbations which are only detectable by subtle metabolic changes as strong compensatory effects in the metabolic system can keep the organism in an apparently healthy state. Metabolomics can thus provide valuable information into the understanding of the biological effects of nanomaterials.

High-throughput metabolomics is also used to evaluate the potential toxicity of nanoparticles, to establish the safety of nanomaterials and to rapidly screen for biomarkers following the nanomedical interventions. Metabolomic analysis can investigate the biological mechanisms and interactions involved in cellular pathways and to identify and validate new cellular targets.

Metabolomics is performed on targeted and untargeted analytical platforms with an expertise in the correct choice of sample types, sample collection procedures, sample analysis (feature detection, normalization, quality control, drift corrections, identification), sample storage and delivery and sample preparation. Furthermore, data processing and statistical analysis are performed well established methods (visualization, uni-/multivariate statistics).

Applications currently include:
- Assessment of energy metabolism
- Detection of metabolites of primary metabolism (amino acids, Krebs cycle, glycolysis, pentosephosphate pathway, CoA, ...)
- Quantification of coenzyme-A activated compounds
- Quantification of spermidine and relatives
- Quantification of certain metabolites
- Metabolite fingerprinting of blood and biological fluids allowing the detection of novel, unknown markers
- Lipid screening

Reference project
MET2NET

Multi-Omics Fusion Technology and Targeted Molecular Imaging: a Fully Integrated Approach for Biomarker Research in Precision Medicine

Multi-omics techniques comprise metabolomics, proteomics, immunolomics, next generation sequencing, digital pathology and MALDI-based applications. Combination with in-vivo molecular imaging provides a worldwide unique setting to direct biomarker research on a nanoscale to early disease detection, patient stratification and therapy response assessment.

As an example, immunolomics is specialized on several immunological assays for both, human in vivo and in vitro studies. Cell culture studies and measurements with material from animal models of type 1 and type 2 diabetes mellitus as well as psoriasis are performed. Pivotal is the understanding of regulatory T-cells in autoimmune disease (e.g. diabetes, irritable bowel disease, rheumatoid arthritis), cancer (e.g. colorectal cancer, leukaemia) and rejection of kidney transplants. Using a state of the art flow cytometer with capabilities of detection of up to 18 markers simultaneously as well as FACS analysis, we are able to perform functional tests with peripheral blood cells from patients with varying diseases (e.g. proliferation assays in co-cultures, ELISpot, ELISA, chemotaxis assays).

In another CoreLab, molecular in-vivo imaging based on radiotracers is coupled to target identification using proteomics. Hence, the visualization and quantification of the in-vivo distribution of specific targets, such as PD-L1, becomes possible which leads to a deeper understanding of underlying molecular mechanisms in malignant diseases. Therefore, we established a workflow allowing for novel target identification and subsequent application in in-vivo imaging using newly developed radiotracers.

Reference project
FFG COMET K1 Centres

CBmed combines – fully integrated multi-omics workflow and in-vivo Imaging.
Atherosclerosis, an inflammatory vascular disease, is among the leading causes of death worldwide. Recent research developments have demonstrated the potential of biomarker-targeted nanosystems for molecular imaging and pharmacological treatment of vulnerable atherosclerotic lesions, which are prone to rupture leading to heart attack or stroke.

Liposomes and protamine-based nanoparticles, called proticles, are promising nanoparticle systems for advanced diagnostic and therapeutic applications. The nanoparticles are synthesized by self-assembling, coating and functionalization with specific targeting moieties, which could be antibodies, peptide/protein sequences or aptamers. As representative examples, the anti-inflammatory proteins interleukin 10 and the globular domain of adiponectin may become efficient biomarkers to target atherosclerotic plaque regions (1, 2). The targeting specificity and accumulation of the nanoparticles in the sites of interest is typically evaluated in cell culture (3) and in vivo/ex vivo in animal models of disease.

Imaging agents, as fluorescence probes, iron oxide nanoparticles (4), gadolinium based complexes or radio-nucleides, are incorporated in the targeted nanoparticles for imaging and detection using either fluorescence microscopy, magnetic resonance imaging (MRI) or nuclear imaging techniques.

The encapsulation of therapeutics in the interior of the targeted nanoparticles is an additional option to achieve selective drug delivery to tissues of interest with the goal to improve drug efficiency and therapeutic intervention of atherosclerosis.

Reference projects and collaborations
FWF/FFG NANO-Health
FP7 NANOATHERO

Reference publications

Nanoprobe-Based Mix & Measure Immunodiagnostics

In collaboration with international partners within the scope of a European project, we have developed a novel approach to immunodiagnostics. It is based on adding specially designed functionalized magnetic nanorods (“nanoprobes”) directly to the sample solution (e.g. serum or saliva), and monitoring their dynamic response to applied time-varying magnetic fields.

When target proteins bind to the nanoprobes, this dynamic response is altered due to the increased hydrodynamic volume of the nanoprobes, which allows to directly quantify the concentration of target proteins in the sample solution. This simple mix & measure approach allows fast and sensitive monitoring of patient parameters without requiring any further manual user interaction.

The main innovative features of our technology are:
(1) Only minimal sample preparation required (e.g. dilution).
(2) Fast total analysis time (< 5 min) due to simple mix & measure technique.
(3) Small sample volumes (20µl currently, 1µl in development).
(4) Good sensitivity compatible to state-of-the-art techniques.
(5) Easy to integrate and simple instrumentation.

Reference project
FP7 NAMDIATREAM

Reference publication

Targeted Nanoparticles for Medical Imaging of Atherosclerotic Plaques

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Reference projects and collaborations
FWF/FFG NANO-Health
FP7 NANO-ATHERO

Reference publications
The success in developing more efficacious future medicines heavily depends on the identification of new disease specific molecular targets, improvements of the delivery technology, and a more detailed understanding of biological processes and molecular mechanisms that govern targeted delivery.

Liposomes are biocompatible, biodegradable and well-established nano-sized vesicles with low toxicity that are increasingly developed as pharmaceutical controlled release formulations. Advanced liposome nanocarriers are typically coated with synthetic polymers and functionalized with targeting ligands, such as peptides, proteins, antibodies or aptamers, which ideally have a high affinity to the target cell or tissue. Liposomes offer the opportunity to encapsulate a high payload of therapeutics or active compounds. Loading of drugs into liposomes can alter the biodistribution profile of the drug, decrease its cytotoxicity and increase the in vivo stability of the therapeutics thereby enhancing the efficacy of the drug by selective transport to pathological sites. Liposomes can be specifically designed to overcome biological barriers and they can be administered by different routes to deliver high drug concentrations directly to disease sites.

Therapeutic liposomes developed include:
- Thiochitosan-coated liposomes with enhanced muco-adhesive properties for oral drug delivery.
- Sterically stabilized liposomes as drug carriers for pulmonary application via inhalation.
- Peptide targeted liposomes for cancer therapy.
- Cationic liposomes for microRNA delivery to adipocytes.
- Liposomes targeted by apolipoprotein mimetic peptides for the treatment of lipid derived chronic inflammatory diseases.

Reference projects
- FWF/FFG NANO-Health
- HTI NANO-FAT
- Ludwig Boltzmann Institute (LBI) Lung Vascular Research
The most relevant and overarching goal in today’s life sciences is to address unmet medical needs and to improve outcomes for patients. Due to scientific advances of the past decade the number of potent drug candidates in development has increased dramatically, together with the opportunity to design novel treatments. However, new “designer-made” drug molecules are becoming increasingly complex, and as a consequence they are more lipophilic and less soluble in aqueous media. Moreover, new biological entities, such as proteins and antibodies are prone to enzymatic degradation and are expected to be inactivated, when exposed to the harsh human physiological conditions. As a consequence, these substances are mainly administered via the parenteral route, which is associated with pain and anxiety, leading to poor patient compliance. Thus, from the perspective of patients and health systems, novel methods to convert complex molecules into effective medicines should be one of the most important objectives, creating an enabling platform technology that will give patients access to many new and innovative drugs that would otherwise never see the light of the day in terms of their application for mankind.

One highly promising technological approach in this rapidly emerging field is particle size reduction to the nanoscale, where solubility enhancement is achieved by making use of the high surface-to-volume ratio and the associated increase in energy levels, and crystal disorder. However, nano-systems need to be converted into safe and effective high-quality medicines (e.g., tablet, capsule). Today, such processes are conventional and batch-based. This is time-consuming, cost-intensive and hard to control. Based on that we developed a continuous in-line controlled hot melt extrusion process for nano-systems, referred to as NANOExtrusion or NANEX. In short, this process converts a stabilized aqueous nano-suspension into a solid oral formulation in a single step, thereby increasing the drug’s solubility, enhancing patient compliance, enhancing process safety, precisely controlling the product quality and reducing development costs.

The process is highly flexible and can be applied to produce a wide variety of different nano-based drug molecules, allowing the development of medicines even for highly insoluble molecules. Thus, NANEX is an enabling platform that can revolutionize the development of complex drug systems, by reducing risks and costs of development significantly accelerating market launch.

Reference projects
FFG NanoExtrusion

Reference publications
How can we improve the treatment of allergies, a global health threat of increasing concern?

Allergen-specific immunotherapy is a procedure, where allergen is applied in increasing doses to the patient to achieve immunomodulation away from allergy. Today, therapies take 3-5 years, resulting in a bad patient compliance. Therefore, our research since many years focuses on the establishment of novel allergy vaccines, with improved efficacy and shorter treatment durations. Nano- and microparticles composed of biocompatible materials have proven to be efficient as non-toxic allergen carrier systems for injective and oral applications. We use *in vitro* CaCo2-co-culture cell systems for cellular uptake experiments and effector cells like RBL-cells for stimulation studies. Novel allergy therapeutics can also be tested in *in vivo* models regarding their safety, side effects, biocompatibility and efficacy with focus on immune-modulation. The read-out parameters include antibodies, cytokines, cell populations, as well as anaphylaxis symptoms with a novel non-invasive heat-imaging system for measurement of body temperature and physical activity changes (Cooperation with Biomedical International R+D GmbH, www.biomed.cc).

Reference publications


Investigations on the Tumor-Biology in Aggressive B Cell Lymphomas - Development of Anti-Cancer Therapies for Aggressive B Cell Lymphoma

Aggressive lymphomas are the most common type of lymphoid malignancies. Despite intensive treatment, a third of all patients will experience relapse of disease.

In our first comprehensive study we showed that NR4A1 and NR4A3 - two members of the nuclear orphan receptor family - are down-regulated in the majority of aggressive lymphoma patients. Both members possess tumor suppressive properties by induction of apoptosis in vitro and in vivo. Additionally, we identified non physiological agonists which massively induce both NR4As and cause NR4A mediated apoptosis of lymphoma cells. Currently, we aim to develop derivatives of these agonists to develop agents with an improved anti-tumoral activity.

In our second study we demonstrated that the surface receptor CXCR4, belonging to the chemokine receptor family, is implicated in the bone marrow infiltration process of aggressive lymphomas, thereby causing a more aggressive clinical course. We modified commercially available antagonists and generated a derivate which suppresses lymphoma growth in vitro. We aim to further modify the derivate to optimize its inhibitory effects on lymphoma cell growth.

Reference publications

Reference projects
Investigating the role of nuclear orphan receptor NR4A1 in hematologic malignancies
Investigating the role of nuclear orphan receptor NR4A3 in Myc-driven lymphomagenesis
The use of CXCR4 antagonists for therapeutic targeting of aggressive B-cell lymphomas

Magnetic Nanoparticles for Biomedical Applications: Modeling and Monitoring of in-vivo Distribution

Magnetic nanoparticles (MNP) offer promising applications in medical therapy and non invasive diagnostics. Their location and physical state can be non-invasively controlled by magnetic fields and they are able to act on cellular level. In novel therapeutic approaches, MNPs are used as markers to identify sentinel lymph nodes in surgical tumor removal, as remotely controlled drug carriers in magnetic drug targeting or as heat generators in magnetic hyperthermia. All these approaches require detailed knowledge of the MNP distribution inside the body for their successful development and clinical acceptance, as the MNP distribution determines drug enrichment and heat production.

Two key factors for obtaining this information are the modeling of the in-vivo distributions and the quantitative, non-invasive imaging of the particles. Monitoring includes knowledge of the systemic and organic distribution, intra-tumoral accumulation and biological interactions. For optimizing therapeutic approaches, understanding physical interactions as the effects of the applied magnetic fields and the influence of particle properties are of particular importance.

Magnetorelaxometry Imaging (MRXI) is a novel modality for the non-invasive, quantitative and specific detection of MNP distributions in-vivo. The delayed magnetic response of the MNP to sudden changes of an external magnetic field is measured with highly sensitive sensors (SQUIDs, optically pumped magnetometers). Employing spatially encoded excitation fields, the distribution of the MNP can be quantitatively reconstructed by solving an inverse problem. Furthermore, information about the interaction of the MNP with the biological environment can be obtained. Therewith, MRXI offers high potential for the monitoring of novel MNP based cancer therapies.

Reference projects
DFG-SPP Projekt CoS-MRXI: Compressed Sensing for magnetorelaxometry imaging of magnetic nanoparticles
EURAMET EMPIR Projekt MagNaStand: Towards an ISO standard for magnetic nanoparticles
COST Action Radiomag: Multifunctional Nanoparticles for Magnetic Hyperthermia and Indirect Radiation Therapy

Reference publications
Therapeutics

Drug delivery and drug targeting are disciplines in the field of pharmaceutical technology that aim at facilitating the transport of critical active molecules (such as proteins, peptides and degradation-prone molecules) to their site of action, at appropriate times, in a controlled manner. Currently, one potential area in drug delivery is the tailoring of nano-carriers, since they are small enough to cross biological barriers. Thereby, the oral administration route is still the most common and convenient route followed by pulmonary delivery.

Due to the need of deposition in the airways, particles for inhalation are usually in the micrometer range. New formulations are being developed to use nanoparticles (NPs) for pulmonary delivery. Since permeability of molecularly dissolved drugs is likely not to be comparable with the permeability behavior of NPs, fundamental knowledge regarding the main barriers (associated with the cellular mechanisms) that impact particle uptake is of enormous interest. At the same time, possible cytotoxic effects and effects on intercellular junctions have to be considered, since several studies suggest that NPs may cause injuries to biological systems.

Due to ethical and economic reasons, as well as small sampling capacity, adequate in vivo systems are often not available and/or comparable to human. Hence, modeling of biological barriers by in vitro cell cultivation has received increased attention. One of the major advantages of such in vitro models is that cellular and sub-cellular functions can be studied in a complex system (integrating all important barriers), achieved by co- or triple-cultivation of different human cell lines (1, 2) and using appropriate culture systems (air-liquid interface) and exposure systems (aerosol, application in simulated lung fluid) (3, 4).

Reference projects and collaborations
EU National Support Land Steiermark: Investigations of the oral uptake route of nanostructured materials

FWF Cellular effects of carbon nanotubes

Reference publications


Models to study interactions of nanoparticles with biological barriers.
Reliable tissue sampling and quantification methods are essential to adequately assess therapeutic effects of drugs. One important aspect to fully understand the concept of nanomedicine is the assessment of various effects of nanoparticles in different tissues. Tissue-specific effects of nanoparticles greatly depend on the local nanoparticle concentration. Therefore, reliable sampling methods are essential to quantify nanoparticles.

Open flow microperfusion (OFM) offers reliable sampling and quantification of nanoparticles in different tissues, such as skin, brain and adipose tissue. OFM uses a steel mesh with macroscopic openings in combination with a peristaltic pump in push/pull mode to achieve stable continuous sampling.

The macroscopic openings allow an unhampered exchange of all substances (incl. nanoparticles) between the interstitial fluid and the OFM sample regardless of substance size, lipophilicity, or charge. Thus, OFM samples represent an unfiltered, merely diluted sample of the interstitial tissue fluid.

Ex-vivo studies are used for a first assessment of nanoparticle pharmacokinetics. OFM has been successfully used in freshly explanted human skin to study nanoparticle transport after topical application on the dermis.

For in-vivo studies OFM sampling can be used to study the pharmacokinetics and pharmacodynamics in animals (preclinical) and humans (clinical). Drug/nanoparticle concentrations can be assessed in muscle, skin, brain and adipose tissue. In combination with the latest analytical methods (Orbitrap, Triple-Quad-MS, GC-MS, HPLC, metabolomics) OFM can provide information about the therapeutic effect of nanoparticles on tissue metabolism.

PlaZentaTox: A Human Ex Vivo Placental Transfer Model

Advances in nanotechnology have resulted in the design of effective, safe and tissue-selective nanocarriers for delivering therapeutics to treat malignancies, infections and other diseases. In pregnancy, nanoparticle-based drug formulations may have the potential to selectively target either, the mother, the placenta and/or fetus. The placenta as the connective organ between the mother and the fetus is a highly species specific tissue. Therefore, the human placental substance transfer, as transport of nanomaterials, cannot be pictured by any animal model. Dual perfusion of a single lobule ex vivo represents the only experimental model to study human placental transfer of substances in organized tissue.

Implementation

- To study total substance transfer from the mother to the fetus.
- To investigate the placental tissue uptake of compounds.
- To determine substance transfer kinetics.
- To monitor in line placental vessel pressure and characteristics.

Technology and Characteristics

- Model approach to study the transfer and transport of substances to the fetal circuit in late pregnancy.
- Mimic the human in vivo situation of normal and pathophysiological placentas.
- Dually system with completely separated maternal and fetal circuits.
- Open or closed circuits allow recirculation of medium, thereby answering specific research questions.
- Physiological conditions: constant body temperature and O₂ concentrations in both circuits.
- Online monitoring of tissue viability and composition of maternal/fetal perfusates during the experiment.

Link to OFM

Reference projects

H2020 Smart4Fabry
2BBB

Scheme of the ex vivo placental perfusion method.
The polyester PLGA (poly-d,l-lactide-co-glycolide) is a biodegradable and biocompatible polymer approved by the EMA and FDA for drug delivery purposes. For site specific drug delivery or diagnostic purposes some parameters can be tuned to yield best fitted particles:

**Loading:** The particles can be loaded either with drugs, marker molecules or both. Nanoparticles built up from up to 35% drug are obtained in case of hydrophobic drugs. In contrast, hydrophilic drugs require altered preparation techniques yielding nanoparticles with loadings of a few tenths of percent. Additionally, lowest amounts of lipophilic, highly fluorescent dyes can be incorporated simultaneously to improve detectability with low leakage of the dye. Thus, the particles can be visualized under the fluorescence microscope, quantified in the fluorescence reader or detected in the flow cytometer in case of cell-association.

**Size:** The diameter and the size distribution of the particles can be tailored by choice of the energy input, the volume ratios, as well as the type and amount of stabilizer.

**Surface modification:** The influence of surface charge on tissue-association and uptake can be elucidated by inverting negatively charged PLGA-particles to positive ones by immobilization of polycations omitting destruction of the matrix. For covalent immobilization of ligands at the particle surface, different activation procedures including spacer-technology are available. Until now, carbohydrate binding proteins as cell-targeters, enzymes for substitution therapy as well as Gd-chelates for magnetic resonance imaging have been immobilized. Moreover, the impact of stabilizers on covalent grafting of particles has been examined.

**Surface Modification of PLGA Micro- and Nanoparticles**

The polyester PLGA (poly-d,l-lactide-co-glycolide) is a biodegradable and biocompatible polymer approved by the EMA and FDA for drug delivery purposes. For site specific drug delivery or diagnostic purposes some parameters can be tuned to yield best fitted particles:

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Therapy of Bladder Diseases

Urinary tract infections belong to the most common infectious diseases worldwide and 50% of the female population experience at least one infection in their lifetime. 95% of these infections are caused by uropathogenic Escherichia coli (UPEC) adhering to high-mannose oligosaccharides of the urothelial membrane. Thus, a biomimetic approach using plant-derived, specifically carbohydrate binding proteins to mediate bioadhesion of drug delivery systems at the urothelium is our rationale for developing more efficacious formulations. Co-localisation analysis revealed that wheat germ agglutinin (WGA) from Triticum vulgare reaches the same accumulation sites like FimH-piliated UPEC and might be useful for anchorage of drug delivery systems. As shown in ex-vivo models, carrier-systems such as drug-loaded microparticles with a lectin-corona not only counteract the harsh wash out conditions in the bladder, but also prolong the residence time as well as the release, shorten the diffusional pathway, and protect the drug from hostile environment. Altogether, in case of superficial diseases this cytoadhesive drug delivery system might mediate enhanced bioavailability and efficacy.

When intracellular drug delivery is preferred, such as in case of bladder cancer, smaller and cell penetrating drug delivery systems are required. Drug-loaded, lectin-grafted nanoparticles or soluble prodrugs consisting of a polymer backbone decorated with drug molecules and lectin targeters represent a promising strategy to take advantage of the cytoinvasive properties of WGA to overcome the urothelial barrier.

All in all, biomimicry of FimH–adhesion represents a platform technology that can considerably improve the therapeutic outcome in the treatment of bladder diseases.

Reference publications


Confirmation of covalent immobilization of rhodamine-labelled protein (rhWGA) on BODIPY-loaded particles (BOD) by colocalisation analysis (overlay).


Colocalization analysis of fLCA and αWGA after binding (4°C) and internalization (37°C) on primary porcine urothelial cell monolayers. Colocalization was evaluated on basis of the Pearson’s correlation coefficient (PCC) and Manders’ colocalization coefficient (M). Scale bar represents 20 µm.
Macro-Molecular Crowding (MMC): A New Approach to Mimic Vocal Fold Scar Under In-Vitro Conditions

Vocal fold fibrosis represents a major disease burden. Screening of antifibrotic compounds could be facilitated by an in vitro fibrogenesis system. However, the development of a fibrogenesis model bears several biological and technical obstacles. Perhaps the biggest challenge is to guarantee a sufficient in vitro deposition of collagen and its incorporation into a pericellular matrix. Fibrogenic cells in monolayer culture do not lay down significant amounts of collagen in an appropriate time for testing antifibrotic compounds. This is due to the fact that cells under conventional conditions are in an aqueous environment that is hardly representative of its in vivo microenvironment. Limitations of existing models might be overcome by implication of the excluded volume effect. This biophysical effect is created by additional macromolecules that occupy a given volume, thereby confining other molecules to the remaining space. The biophysical implications of this effect have consequences on reaction kinetics and molecular assembly. Macromolecules drive reaction partners into closer collaboration, resulting in improved protein-folding and protein–protein interactions. Our studies demonstrated that MMC is a suitable approach to enhance collagen-alpha1(I) deposition in cultured rats’ vocal fold fibroblasts. Collagen-alpha1(I) deposition increased significantly under crowded conditions in supernatant (up to 9-fold) as well as in the cell layer (up to 4-fold). Levels of numerous other important ECM components (HA, fibronectin, collagen-III) were also found to be increased. This model has the potential for fast and effective in vitro collagen deposition and may provide a valid alternative to animal experiments and can be transferred to any other type of fibroblast.

Reference project

Construction of autologous bioengineered laryngeal mucosa (funded by Jubiläumsfonds OeNB)

Immunocytochemistry for collagen I: (b): uncrowded state, (d): crowded state, scale bar: 100 µm.
Skin Wound Healing – Skin Regeneration, Repair and Reconstruction

Skin Wound Healing is a physiologic response to tissue injury and tissue damage; it is the complex interaction of different processes that allow resurfacing, reconstitution, and restoration of the injured skin. Healing is a systematic process, traditionally explained in terms of 4 overlapping classic phases: hemostasis, inflammation, proliferation, and maturation.

The expertise of our research group is to perform all kind of research projects within the field of skin wound healing and wound care.

- Our research group is dealing with the topic Tissue Engineering (replacing and engineering of cells and tissues) and
- with the topic of stimulating the body’s own repair mechanisms (to regenerate human cells and tissues) in order to restore or establish normal function.

One of the main research areas is to develop and prove new products and treatment concepts in order to improve wound healing and thereby the functional outcome.

- Based on our expertise and research results we try to support a fast translation of research into the clinical routine in order to improve the therapeutic options for patients suffering from wounds and impaired wound healing. (This fast translation from ideas into the clinical routine is possible due to a profound interlocking of industry, basic research and clinical research: Prometheus Network).

Within the field of wound healing and wound care we are experienced in planning and performing the whole spectrum of projects and trials (from in-vitro -> ex-vivo -> in-vivo).

- The main focus of our research group is to develop new and/or to improve existing surgical and non-surgical treatment options in the field of wound care based on profound basic and clinical research.

Reference network
Prometheus
www.prometheus-netzwerk.at

Reference publications
(3) Yanai H, et al. Middle age has a significant impact on gene expression during skin wound healing in male mice. Biogerontology. 17(4), 763-70 (2016).

HREM (High-resolution episcopic microscopy) image of normal skin and its vascular architecture.