Med Uni Graz *Pioneering Minds*

Biomedical Research

ALTERNATIVE APPROACHES TO ANIMAL TESTING AT THE MEDICAL UNIVERSITY OF GRAZ

CONTENT

CELL & TISSUE CULTURE

CELLBANK

CellBank Graz offers a wide range of services and competent technical support for cell and tissue cultures. The ISO certification of the Core Facility guarantees comprehensive quality control of cell lines in the form of myocoplasma tests and cell authentication tests using STR analyses, as well as a controlled standardized storage system.

Established patient-derived cell lines from various tumor entities are available to researchers according to the highest quality standards and detailed characterization.

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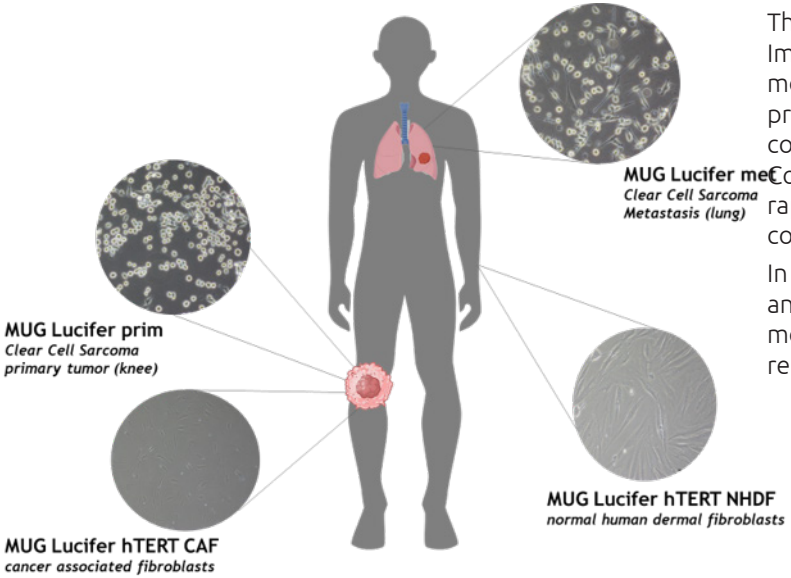
AUTOLOGOUS TUMOR MODELS



Different cells isolated from a patient to establish co-culture models.

The Core Facility Alternative Biomodels & Preclinical Imaging specializes in establishing adequate cell culture models (primary culture, 3D, and co-culture). Due to the proximity to the University Hospital Graz, the excellent cooperation between the various disciplines and the Comprehensive Cancer Center, efficient tissue transfer, rapid isolation of cells, reduced ischemia time, and comprehensive know-how are guaranteed.

In addition, the Core Facility offers a cytostatic bench, an automated liquid handling system, and continuous monitoring of cell proliferation, migration, and invasion in real-time.

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BARRIER MODELS

The body and its organs are protected from uncontrolled infiltration of substances by epithelial barriers. Outer barriers avoid contact with the environment (epidermis, cornea, as well as respiratory, oral and urogenital mucosae). Inner barriers separate inner organs and blood and exist between lymphatic organs, reproductive organs and brain. Permeation and inflammation at barriers can be assessed by *in vitro* models.

Respiratory Tract

Calu-3 cells on membranes cultured at an air-liquid interface culture

Intestinal Tract

Caco-2 cells on membrane inserts – with and without mucus-overlay

Blood-Brain-Barrier

Static : hcMEC/D3 + primary astrocytes on membranes
Dynamic: CELLMAX® DUO bioreactor

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SMALL INTESTINAL IN VITRO MODEL

The intestinal tract is one of the most complex organs and contains the greatest number and diversity of immune cells in the body. Several subsets of mononuclear phagocytes and DCs (MDC) populate the small intestine (SI), and these cells reportedly exert specialized functions in anti-microbial immunity and tolerance.

In order to understand respective activities of these cells in the mucosal landscape, we established a new model which realistically recapitulates the cellular composition of the human SI comprising epithelial cell lines and diverse immune cell subsets, including intestinal mononuclear phagocytes, which have collectively emerged as key players in the maintenance of gut homeostasis, the development of gut inflammation and as well its resolution. In depth understanding of specific roles of these cells in the small intestinal immune landscape might pave the way for novel treatments of inflammatory disorders (like Crohn's disease or ulcerative colitis).

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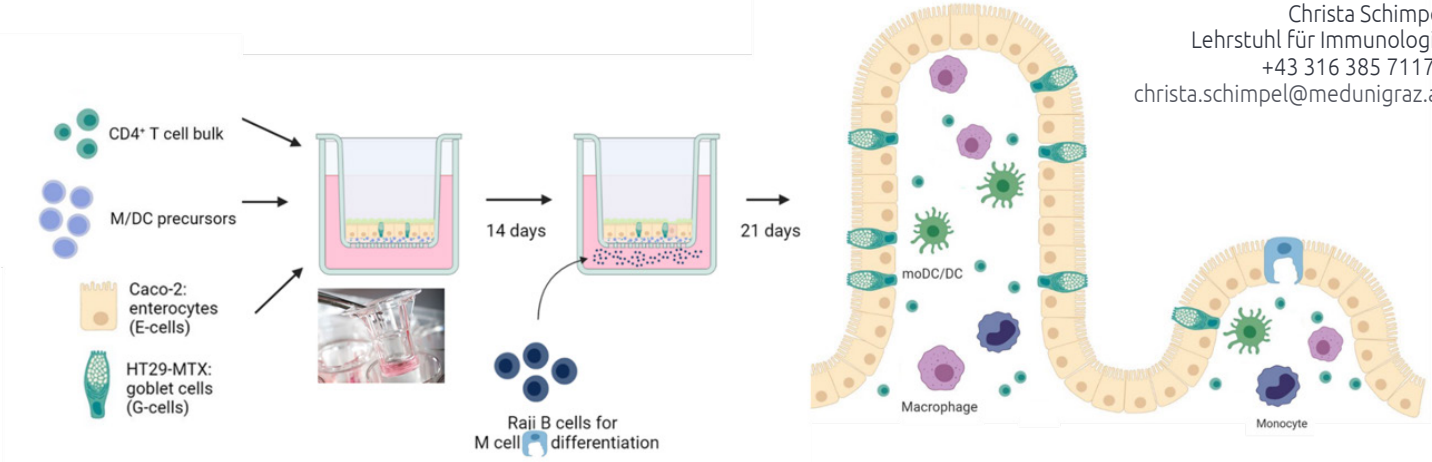


ORGAN-ON-A-CHIP: LET IT FLOW!

The organ-on a chip technology addresses increasing demands of physiology in vitro – including flow !

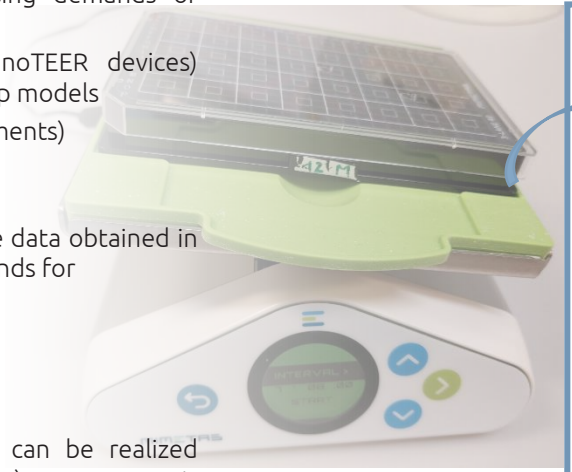
The Mimetas Organoplate system (including OrganoTEER devices) provides the generation of miniature 3D organ-on a chip models

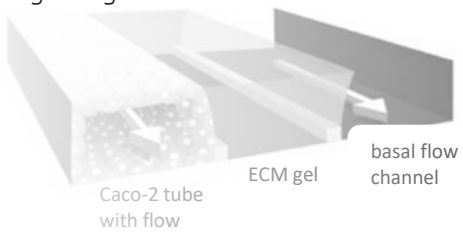
- lacking artificial membranes (as separating compartments)
- enabling high troughput
- and most important - including physiological flow !

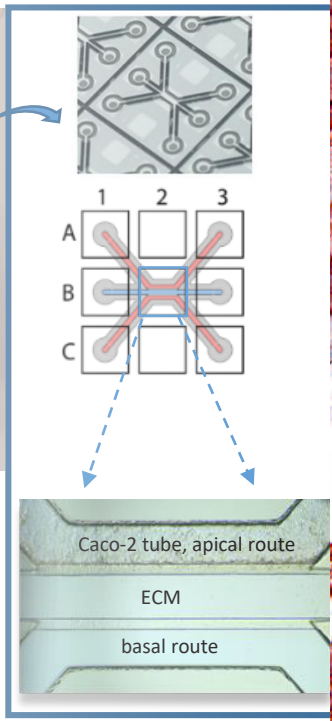
It represents state-of-the-art technology to re-evaluate data obtained in 2D cell culture and can be used to address e.g. compounds for

- toxicity-related aspects
- potential protective effects
- effects on (intestinal) barrier function
- and many more.

Several models with different levels of complexity can be realized (immortalized cell lines, co-culture, organoid-on a chip…) as e.g. our gut-on-a chip models (for normal/leaky gut).







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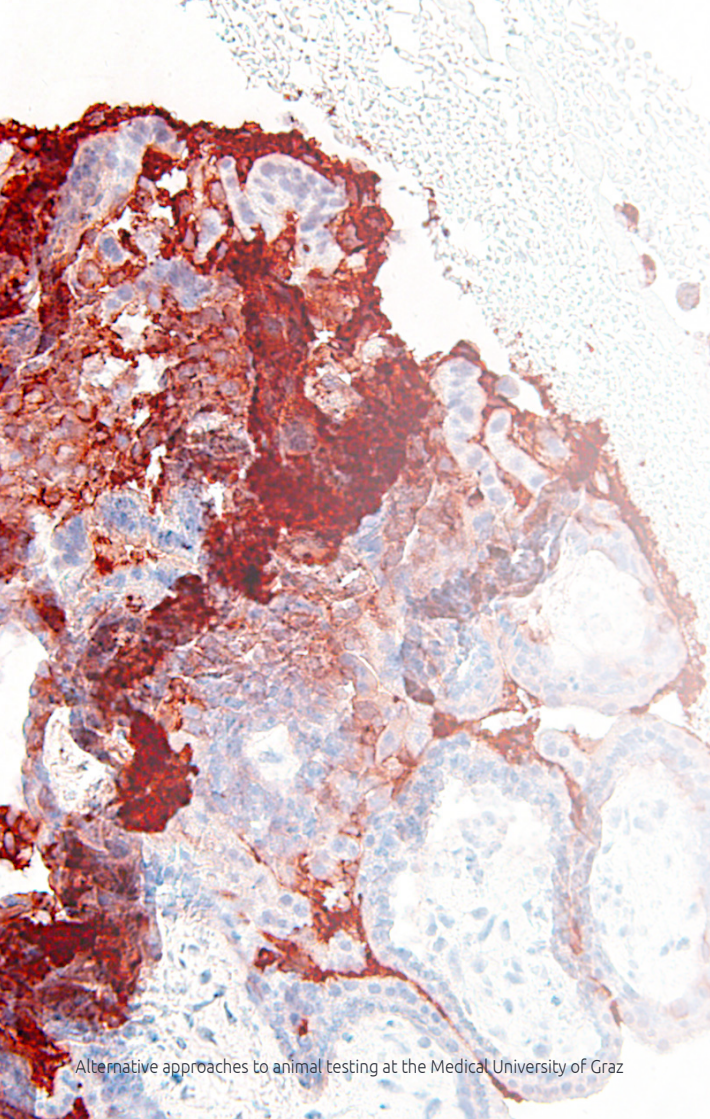

PERFUSED 3D TISSUE CULTURE

The 3D organ lab model helps to investigate biochemical processes and their pathologies in complete organs. This model combines the following advantages: 1) Different cell types are composed 2) in a 3D network and could be cultivated 3) in defined compartments 4) with diverse physical and chemical conditions, wherein 5) direct cell-to-cell contact can be promoted or avoided. This model consists of an electrospun hollow fiber of polycaprolactone modified with polylactide (PCL/PLA). The hollow fiber is linked with luer lock connections in a polycarbonate housing to create two discriminative compartments: the inner surface facing the lumen, representing the inner compartment and a second (outer) compartment. Both can be seeded with cells from various tissues. The PCL/PLA mesh allows a cross-talk of cells by the exchange of paracrine factors or could offer a direct cell-to-cell contact. The cells in the inner and outer compartment are applied to conditions appropriate to their requirements, e.g. flow, nutritional supplies, or variations in the oxygen concentration. The following models are already established and ready-to-use: blood vessel, trachea/ bronchus, intestine, placenta (invasion model).

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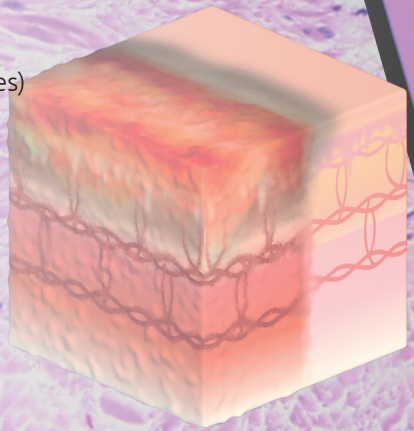
EX VIVO SKIN MODELS

Human ex vivo skin model for short and long-term experiments

- Complementary to preclinical and clinical studies
- Histologic and molecular characterisation of biopsies
- Open flow microperfusion (OFM): Biomarker analysis (in cooperation with JOANNEUM RESEARCH)

- Mimicking burn injury
- Skin inflammation
- Biomarker mobilization (miRNAs, cytokines)

- Wound healing and Scarring
- Inflammation
- Treatment validation



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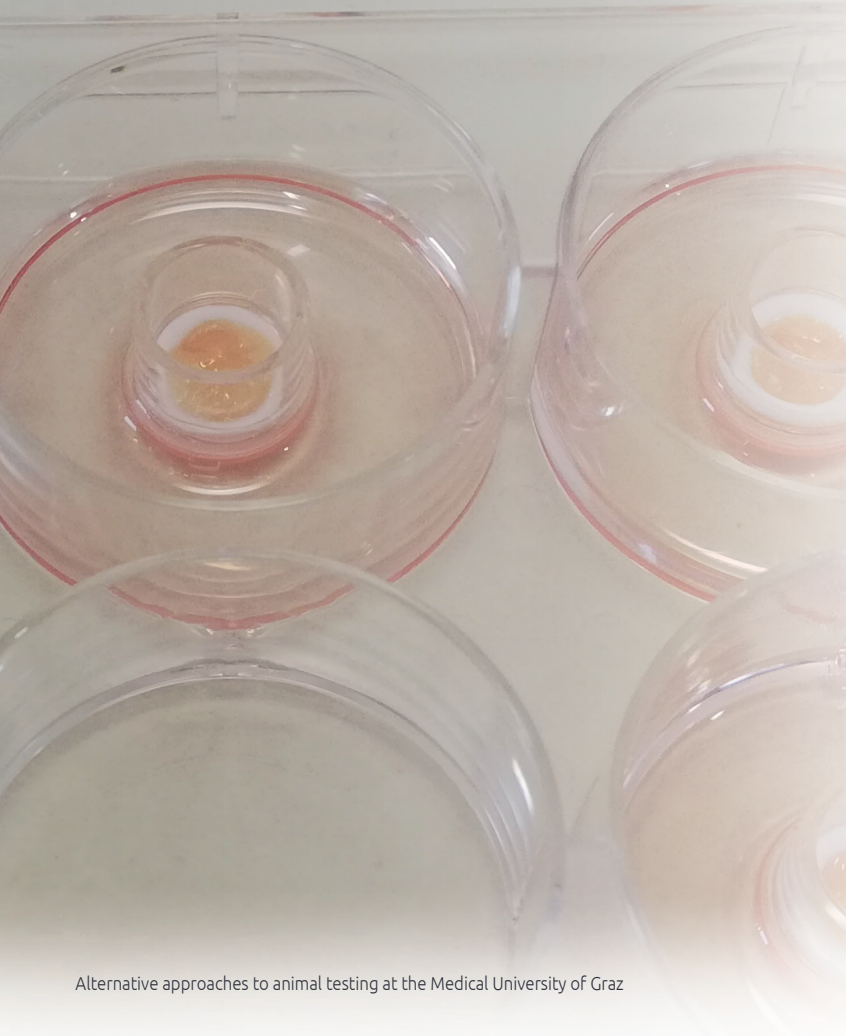

HUMAN JUVENILE 3-D SKIN MODELS

3-D Models with various levels of complexity

Build-Up Models

from isolated juvenile skin cells > autologous models

- RHE (reconstructed human epidermis)
- SE (skin equivalent)

Ex-Plant Culture

skin punches from juvenile foreskin in defined sizes

- hOSEC (human organotypic skin explant culture)
- culture media without animal dervied supplements
- topical / subcutaneous administration of testing compounds (cremes, chemicals,...)
- analysis of:
  - culture media (cytokine expression/cytotoxicity, …)
  - histological changes (HE staining, specific markers, ...)

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ORGANOTYPIC CULTURES

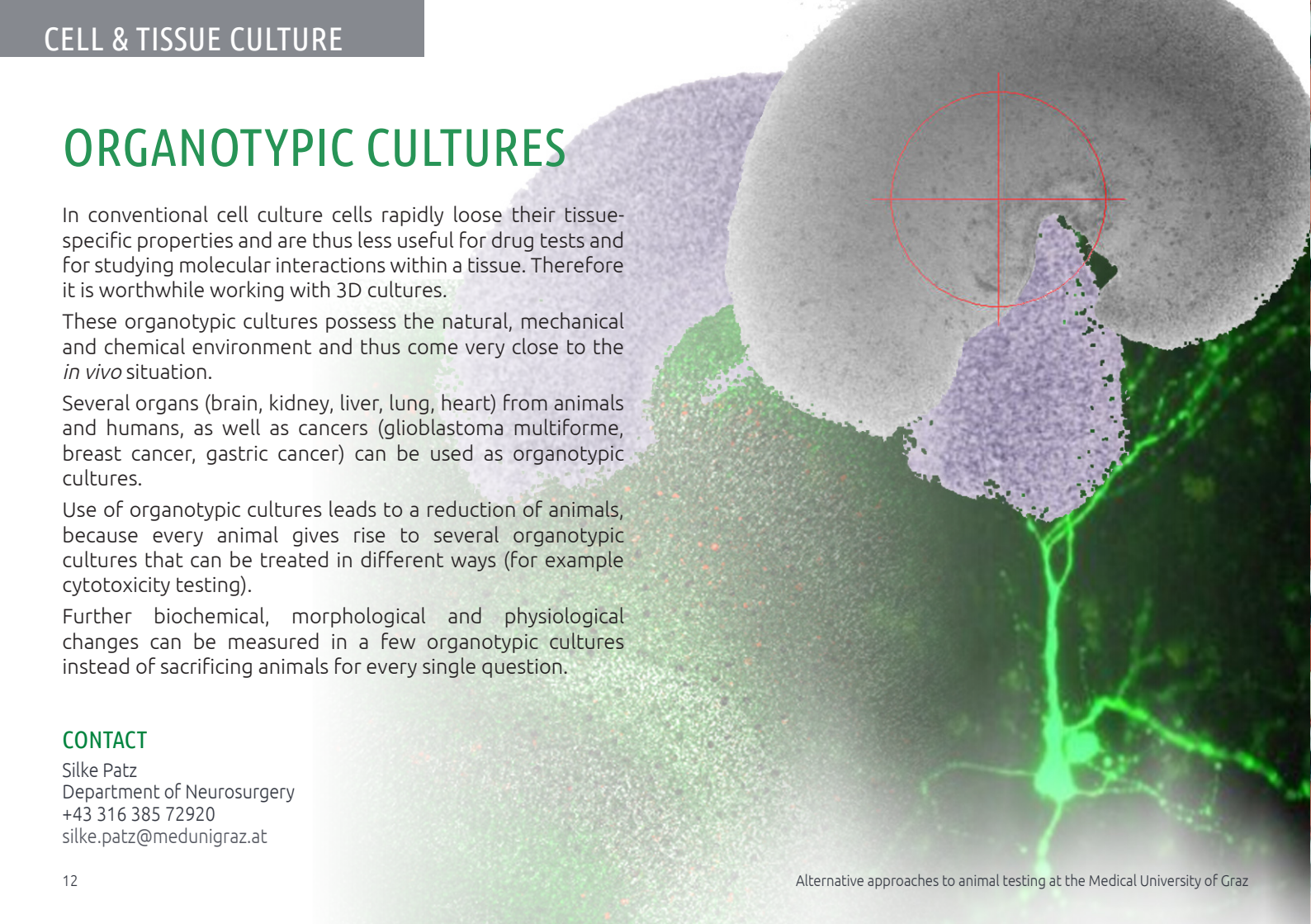
In conventional cell culture cells rapidly loose their tissue-specific properties and are thus less useful for drug tests and for studying molecular interactions within a tissue. Therefore it is worthwhile working with 3D cultures.

These organotypic cultures possess the natural, mechanical and chemical environment and thus come very close to the *in vivo* situation.

Several organs (brain, kidney, liver, lung, heart) from animals and humans, as well as cancers (glioblastoma multiforme, breast cancer, gastric cancer) can be used as organotypic cultures.

Use of organotypic cultures leads to a reduction of animals, because every animal gives rise to several organotypic cultures that can be treated in different ways (for example cytotoxicity testing).

Further biochemical, morphological and physiological changes can be measured in a few organotypic cultures instead of sacrificing animals for every single question.



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BIOBANK GRAZ

Biobank Graz is a central research infrastructure at the Medical University of Graz and coordinates joint biobanking in close collaboration with its clinical partners. Biobank Graz actively collects, processes, stores and delivers high quality biospecimen and associated data to the research community according to national and international legal and ethical standards in biobanking.

The available biological specimen include formalin-fixed paraffin-embedded (FFPE) tissues, cryopreserved tissues and biological fluids. More information can be found on the Biobank Graz webpage: biobank.medunigraz.at

Special attention is paid to sample quality. The facility is ISO 9001:2015 certified. Depending on experimental demands, prospective cohorts are collected according to appropriate CEN/ISO standards.

Services of Biobank Graz:

- Provision of retrospective samples and data
- Project development and implementation
- Planning and realization of prospective study cohorts
- Integration of pre-existing collections into Biobank Graz

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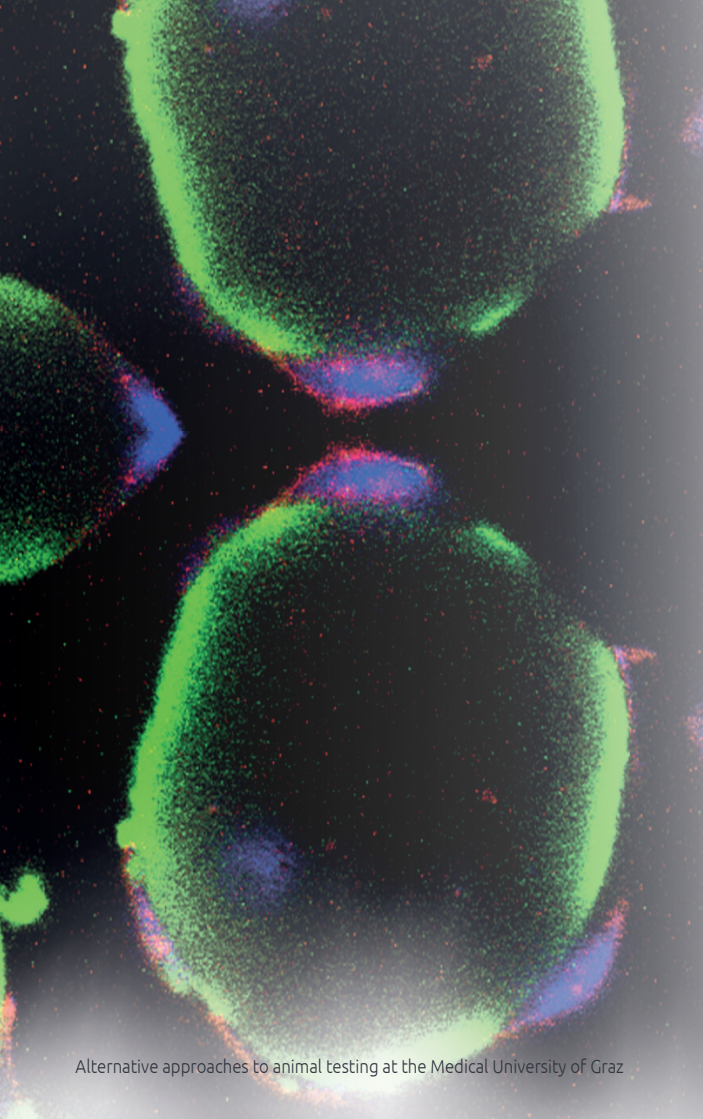
ALTERNATIVE MODELS



CELL-BASED TOXICITY ASSAYS

Cell-based toxicity assays offer a wide range of possibilities to assess important hallmarks in drug discovery processes.

Genotoxicity

Assays for
- Mutagenesis
- Clastogenic and aneugenic changes
- DNA repair mechanism

Hemocompatibility

Assays for
- Hemolysis
- Plasmatic coagulation
- Platelet activation
- Complement activation

Immunotoxicity

Assays for
- Phagocyte function (NO generation, respiratory burst, chemotaxis, phagocytosis, surface markers)
- Panels of cytokines

Chronic Cytotoxicity

3D culture of cells on
- Microbeads in benchtop biorector
- Membranes at an air-liquid interface

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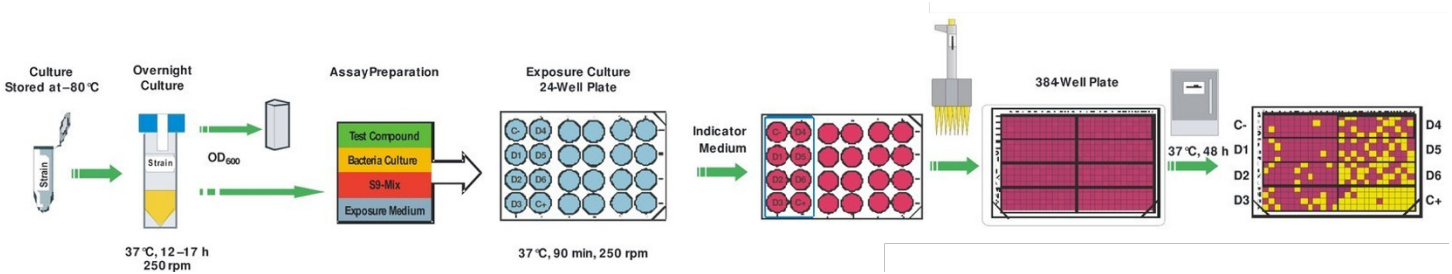
BIOTESTING AND CYTOTOXICITY

Biological mutagenicity and cytotoxicity assays can assess the hazardous potential of complex mixtures or chemicals in a very timely manner. Therefore, studies with an appropriate combination of these assays can provide useful information about the toxic/mutagenic potential.

- AMES ASSAY
- UMU ASSAY
- CALAX ASSAY

AMES ASSAY

The Ames Assay is a fast and widely employed bacterial tool to screen for mutagenic effects of chemicals or extracts and can be performed with S9 (crude liver enzyme extract) for metabolic activation. It allows for the detection of base-pair substitutions and frame-shift mutations.



Ames Assay procedure (image courtesy of hjs consulting, Germany)

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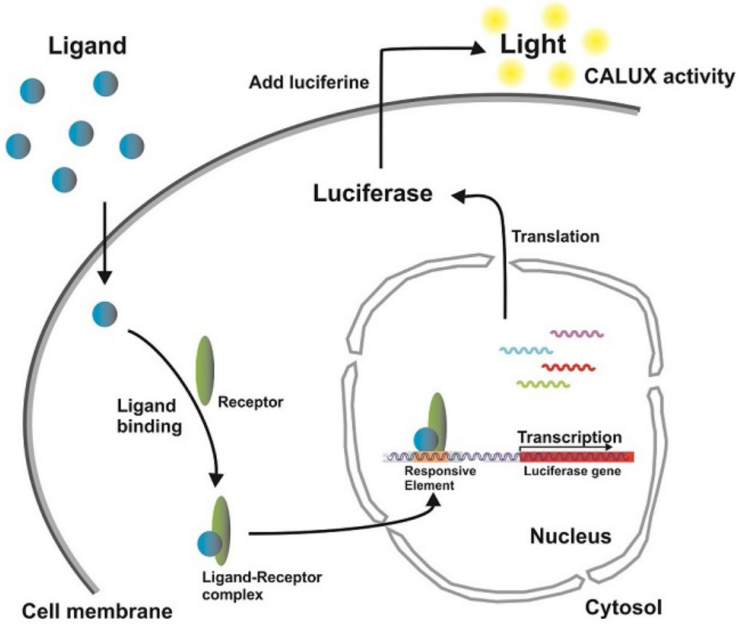

CALUX Assay principle
(image courtesy of BioDetection Systems bv, Netherlands)

UMU ASSAY

The Umu Assay is a lesser known bacterial tool for assessment of mutagenic and cytotoxic potential with or without S9, which is based on the ability of mutagens to induce genes involved in bacterial mutagenesis via the SOS-repair pathway and is performed in *S. typhimurium*.

CALUX® ASSAY

The CALUX® (Chemical Activated Luciferase gene eXpression) Assays (BioDetection Systems bv, Netherlands) allow for sample testing in an eukaryotic system with a quantifiable response. The CALUX Assays use the human bone osteosarcoma cell line U-2 OS allowing for testing for genotoxicity (p53 CALUX), endocrine disruptors ((anti-)ERα CALUX, (anti-)AR CALUX) as well as cytotoxicity (Cytotox CALUX).

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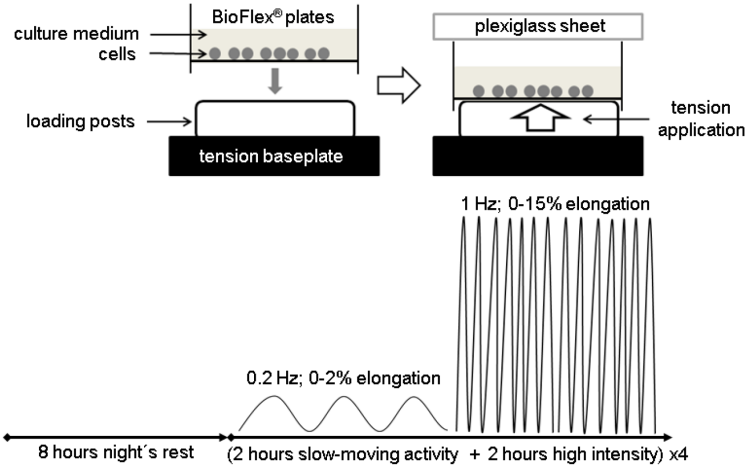
FLEXCELL® FX5K™ TENSION SYSTEM

The Flexcell® FX5K™ Tension System functions as a computer-controlled bioreactor designed to subject cells cultured in vitro to cyclic or static tensile strains. This system utilizes regulated vacuum pressure to alter flexible-bottomed culture plates, resulting in a substrate elongation of up to 25%. It has the capability to program multiple changes in frequency, amplitude, and waveform within a single regimen, effectively replicating tissue strains and frequencies found in vivo in various cell types including muscle, lung, heart, blood vessels, skin, tendon, ligament, cartilage, and bone.

A range of waveform options such as static, sinusoidal, heart stimulation, triangular, square, and customizable are available. The mechanical stimulation is carried out through four independent FlexLink remote compression and/or tension controllers designed for 6-well sized plates.

The BioFlex® Culture Plates possess optical clarity, allowing for direct observation of cells using both inverted and upright microscopes. These plates offer a total growth surface area of 57.75 cm2 and come with a variety of available matrix surfaces including Amino, Collagen (Type I or IV), Elastin, Pronectin® (RGD), and Laminin (YIGSR). In addition, the Tissue Train® 3D culture system allows users to create 3-dimensional cell-seeded gels and apply uniaxial tensile strains to these gels.



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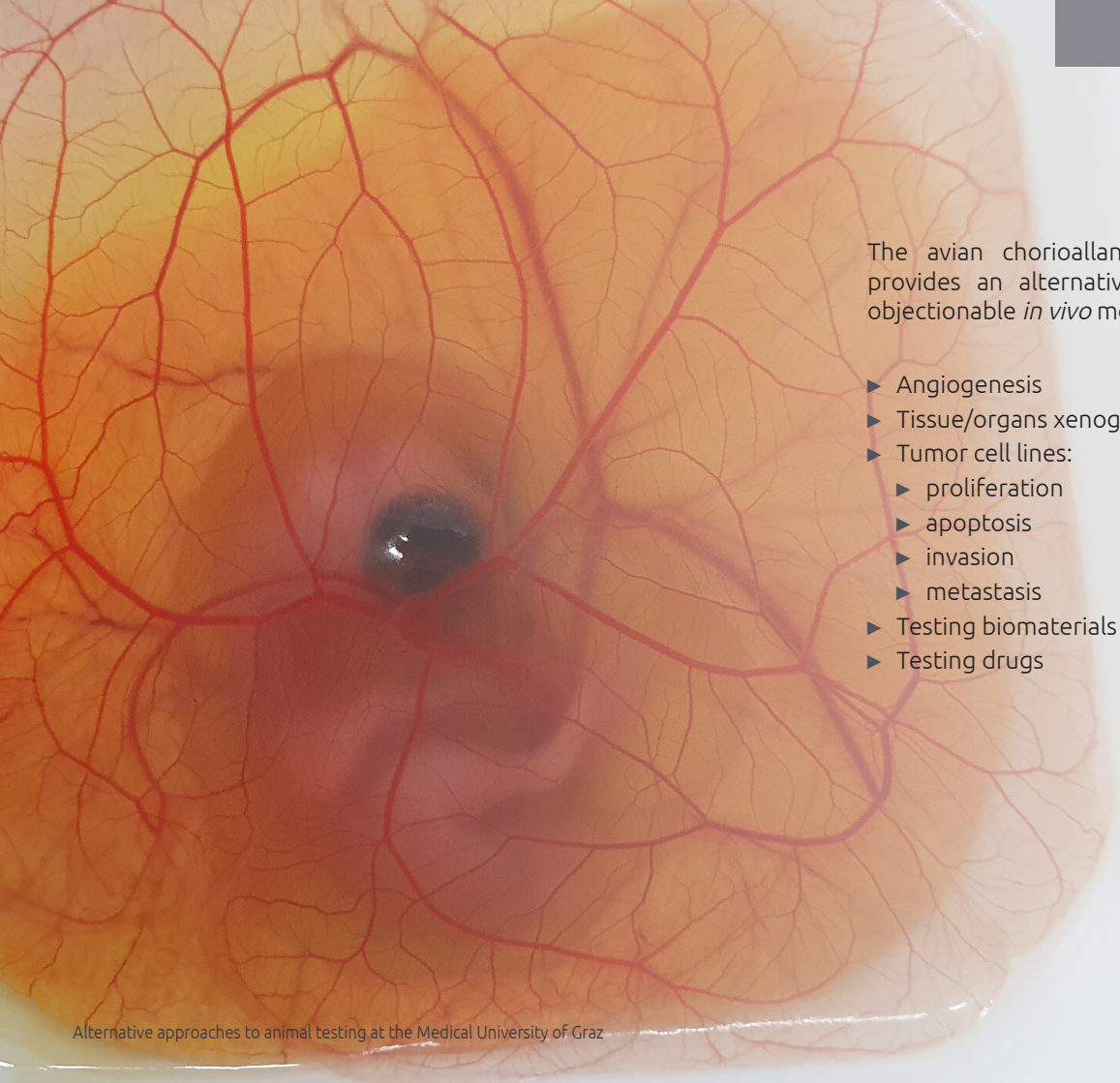

CAM ASSAY

The avian chorioallantoic membrane (CAM) assay provides an alternative versatile and ethically less objectionable *in vivo* model for many applications:

- Angiogenesis
- Tissue/organs xenografts
- Tumor cell lines:
  - proliferation
  - apoptosis
  - invasion
  - metastasis
- Testing biomaterials
- Testing drugs

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PERFUSION OF THE HUMAN PLACENTA

Spatio-temporal development of pregnancy and placenta in animals differs substantially from human. Results from animal models to study transfer of substances across the placenta lack significance. The *ex vivo* perfusion of a single human placental lobule is the most reliable experimental model.

Technology

- Intact human tissue, different biological barriers
- To investigate vasoactive characteristics of drugs in a human vascular system
- To determine uptake, efflux and transfer of substances across distinct barriers
- To study transfer kinetics of substances and metabolites
- Easy accessible tissue, no ethical concerns exist

Charactaristics

- A reliable model for the transfer of substances from the mother to the fetus in late pregnancy
- To imitate late placental physiology *ex vivo* over several hours
- A dual system with completely separated maternal/fetal circuits
- Physiological conditions for different circuits: medium, plasma, constant body temperature, pressure control and variable O_2 concentrations
- Placental tissue from different pathophysiologies available

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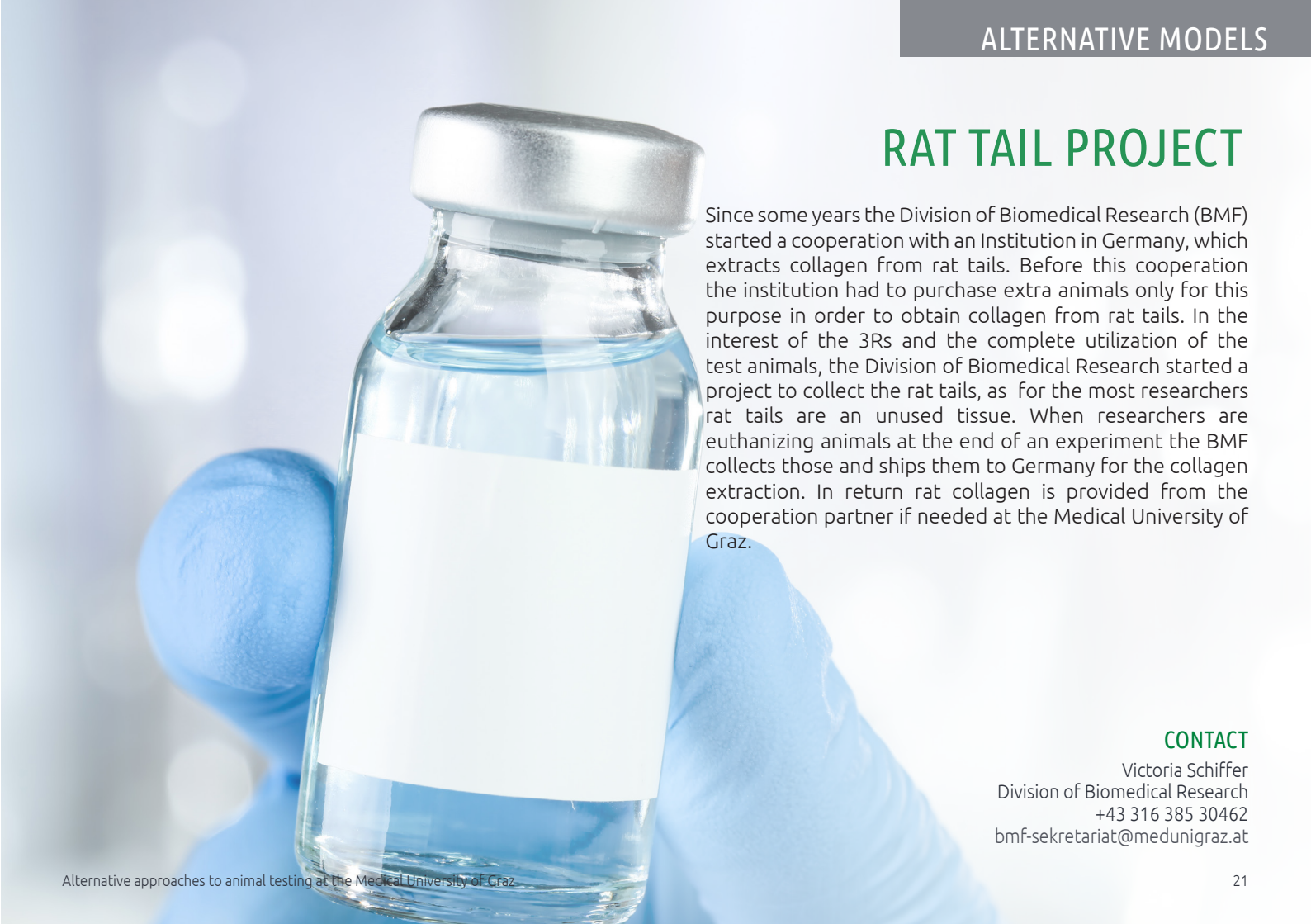

RAT TAIL PROJECT

Since some years the Division of Biomedical Research (BMF) started a cooperation with an Institution in Germany, which extracts collagen from rat tails. Before this cooperation the institution had to purchase extra animals only for this purpose in order to obtain collagen from rat tails. In the interest of the 3Rs and the complete utilization of the test animals, the Division of Biomedical Research started a project to collect the rat tails, as for the most researchers rat tails are an unused tissue. When researchers are euthanizing animals at the end of an experiment the BMF collects those and ships them to Germany for the collagen extraction. In return rat collagen is provided from the cooperation partner if needed at the Medical University of Graz.

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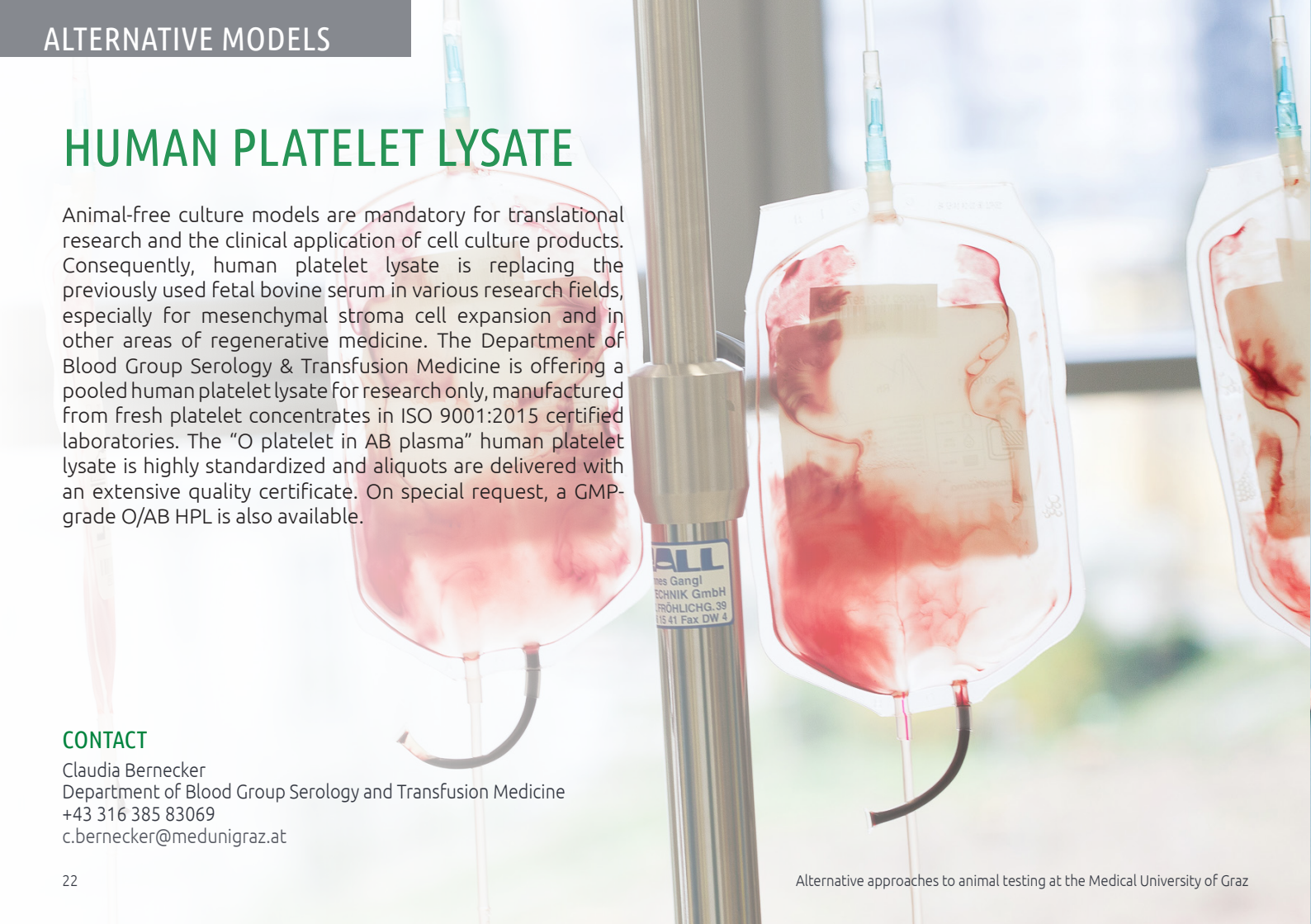
HUMAN PLATELET LYSATE

Animal-free culture models are mandatory for translational research and the clinical application of cell culture products. Consequently, human platelet lysate is replacing the previously used fetal bovine serum in various research fields, especially for mesenchymal stroma cell expansion and in other areas of regenerative medicine. The Department of Blood Group Serology & Transfusion Medicine is offering a pooled human platelet lysate for research only, manufactured from fresh platelet concentrates in ISO 9001:2015 certified laboratories. The "O platelet in AB plasma" human platelet lysate is highly standardized and aliquots are delivered with an extensive quality certificate. On special request, a GMP-grade O/AB HPL is also available.



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DUMMIES INSTEAD OF ANIMALS IN EDUCATION

Dummies like the pig's head, the silicone model of a rabbit ear or rat and mouse dummies help to pursue the targets of reducing the burden (severity level) on the animals by non-experienced persons and the number of animals effectively used by making frequent repetitions of a technique possible.

Techniques like venous blood extraction from the ear (replaceable rubber tube), placing a venous/arterial catheter in the "ear vein"/"artery of the ear", placing an arterial catheter in the common carotid artery or endotracheal intubation can be trained on the pig head dummy.

Practicing blood sampling on rabbits itself is a big burden on the animal. The silicone model of the ear is a proven replacement method to train this technique.

Rat and mouse dummies (entire animal and skeleton dummies), which accurately display the anatomy of the animals can substantially reduce the number of animals used for autopsies.

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IN SILICO MODELLING

EXPERIMENTAL DESIGN AND DATA ANALYSIS

Experimental Design

- Unbiased, adequately powered, with a wide range of applicability, amenable to statistical analysis, simple and efficient designs
- Proper sample size calculation (in accordance with the 3Rs) to produce reliable and predictive results
- Power analysis for given sample sizes and effects of variation in retrospective studies
- Project support from the first concept until the interpretation of your data

Data Analysis

- State-of-the art know how for bioinformatics and statistical data analysis
- Broad range courses focused on bioinformatics and biostatistics (zmf.medunigraz.at/merag)
- Growing number of tools for data processing and analysis
- Access to a platform for reproducible, and transparent computational biological research (galaxy. medunigraz.at)
- Access to a High Performance Computing Cluster (MedBioNode) for comprehensive data analysis

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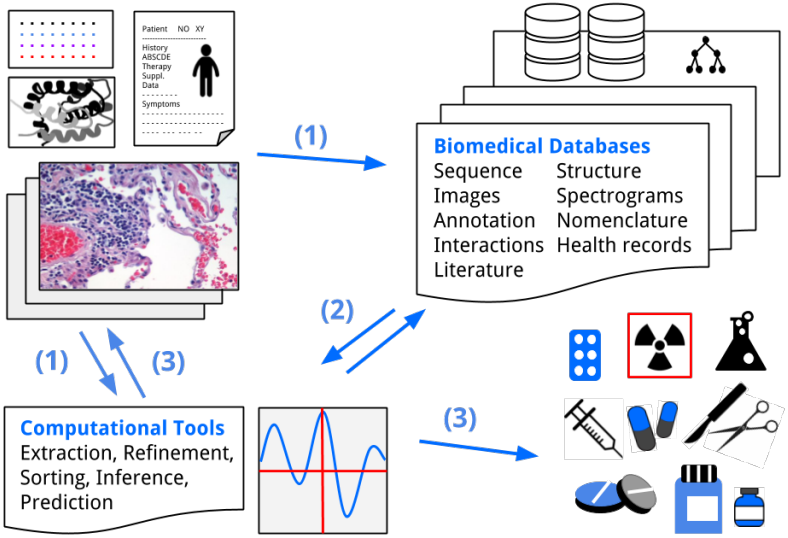
IN SILICO MODELING AND INTEGRATING OPEN DATA

The design development and evaluation of algorithms, methods and tools support to understand the underlying principles of diseases, particularly cancer.

One of the keys to understand the concepts of cancer lies within an integrative translation and multi-dimensional connection of open data sets.

AI/machine learning approaches to biomedical analysis and simulation involve several techniques such as validation, classification, inference, prediction and modeling. Existing well-maintained databases provide, integrate and annotate information on various diseases and are increasingly being used to generate predictive models, which in turn will inform and guide biomedical experiments.



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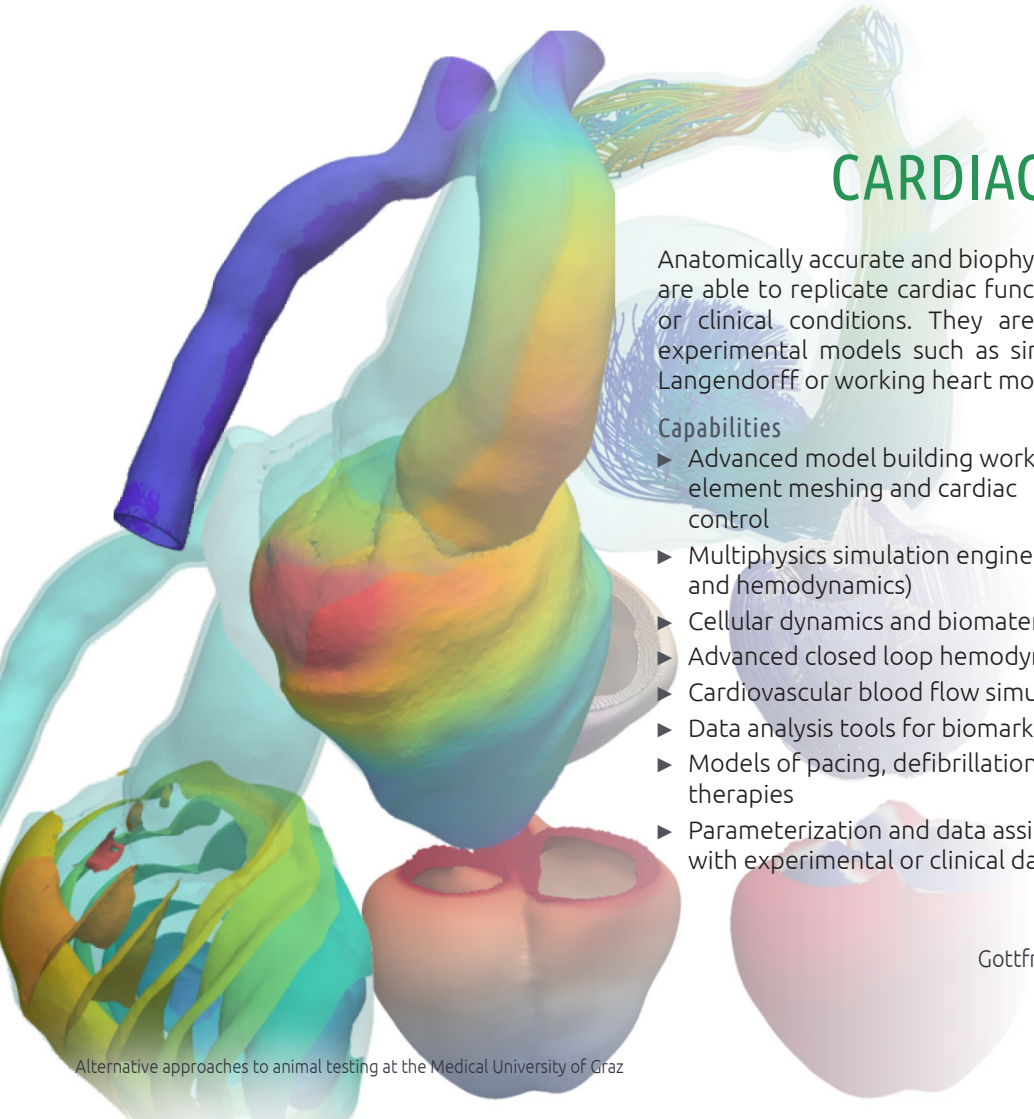

CARDIAC *IN SILICO* MODELS

Anatomically accurate and biophysically detailed *in silico* models of the heart are able to replicate cardiac function under a broad range of experimental or clinical conditions. They are used to complement or even replace experimental models such as single cell patch clamp, tissue monolayers, Langendorff or working heart models.

Capabilities

- Advanced model building workflows comprising image based finite element meshing and cardiac navigation systems for local feature control
- Multiphysics simulation engine (cardiac electrophysiology, biomechanics and hemodynamics)
- Cellular dynamics and biomaterial models for various species
- Advanced closed loop hemodynamic models
- Cardiovascular blood flow simulations
- Data analysis tools for biomarker extraction
- Models of pacing, defibrillation, cardiac resynchronization and valve therapies
- Parameterization and data assimilation techniques to match simulation with experimental or clinical data

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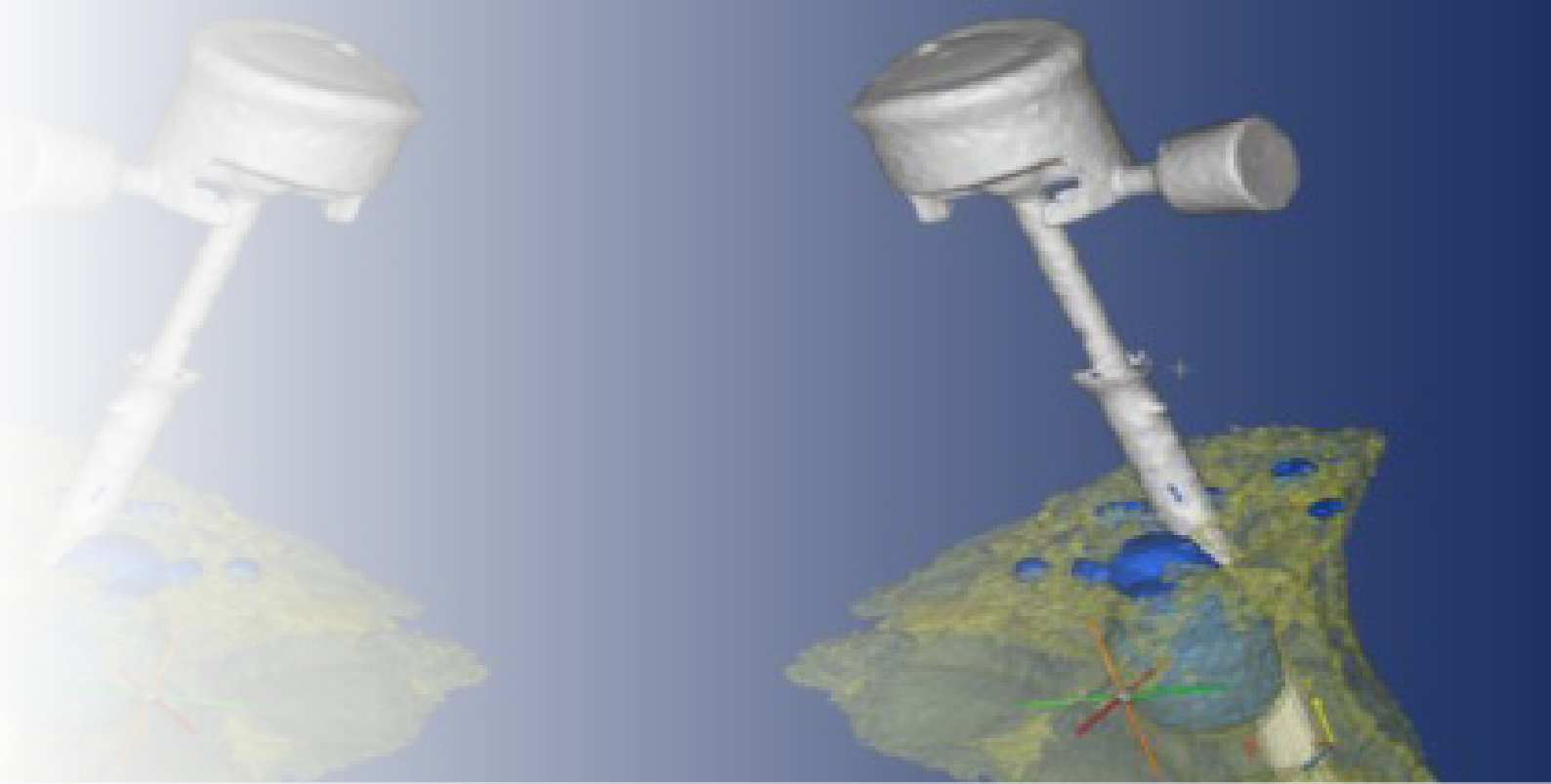


PRECLINICAL IMAGING

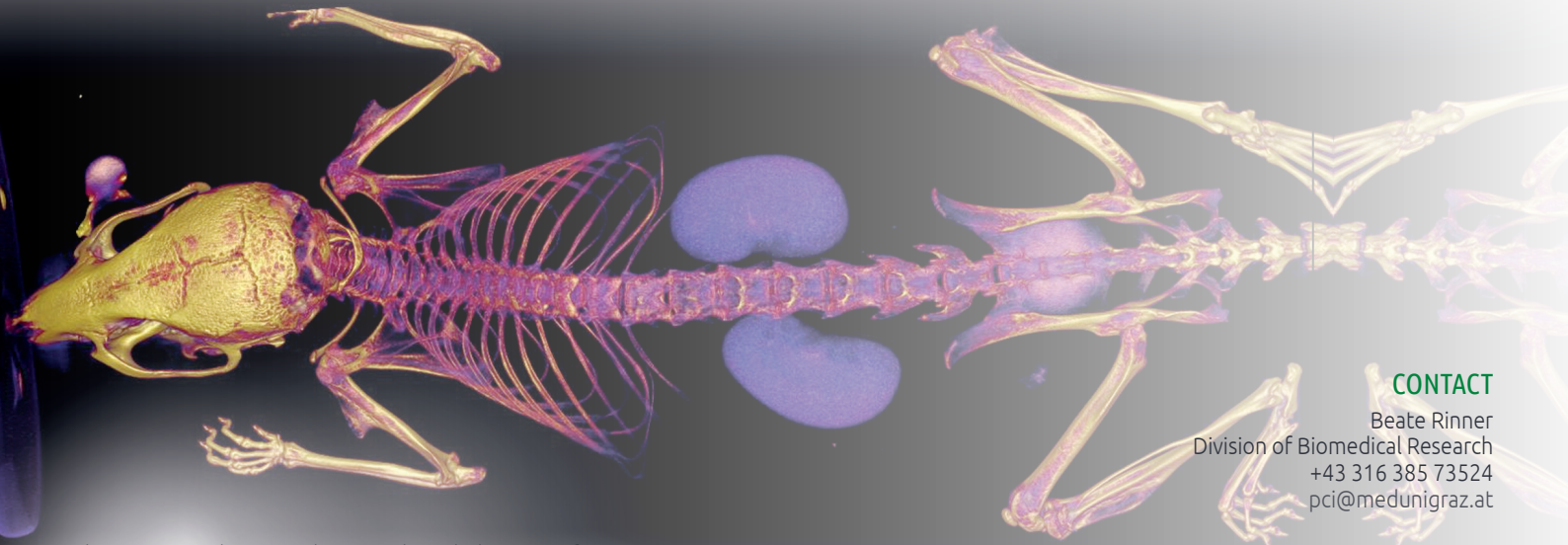
MICRO COMPUTED TOMOGRAPHY

Micro computed tomography (SkyScan 1276© by Bruker) is a high-resolution, non-invasive radiographic imaging technique that can be used for both pathological and physiological analysis *in vivo* as well as for *in vitro* surface and structural analysis of materials and implants. Thanks to well-developed contrast agents, it is possible not only to visualize bones but also to examine soft tissues.

Maximum resolution *in vivo* is 9 micron, for *ex vivo* analysis 5 micron.

Some of the main fields of application are:

- Bone densiometry
- Cancer research
- Whole body fat measurement
- Material analysis



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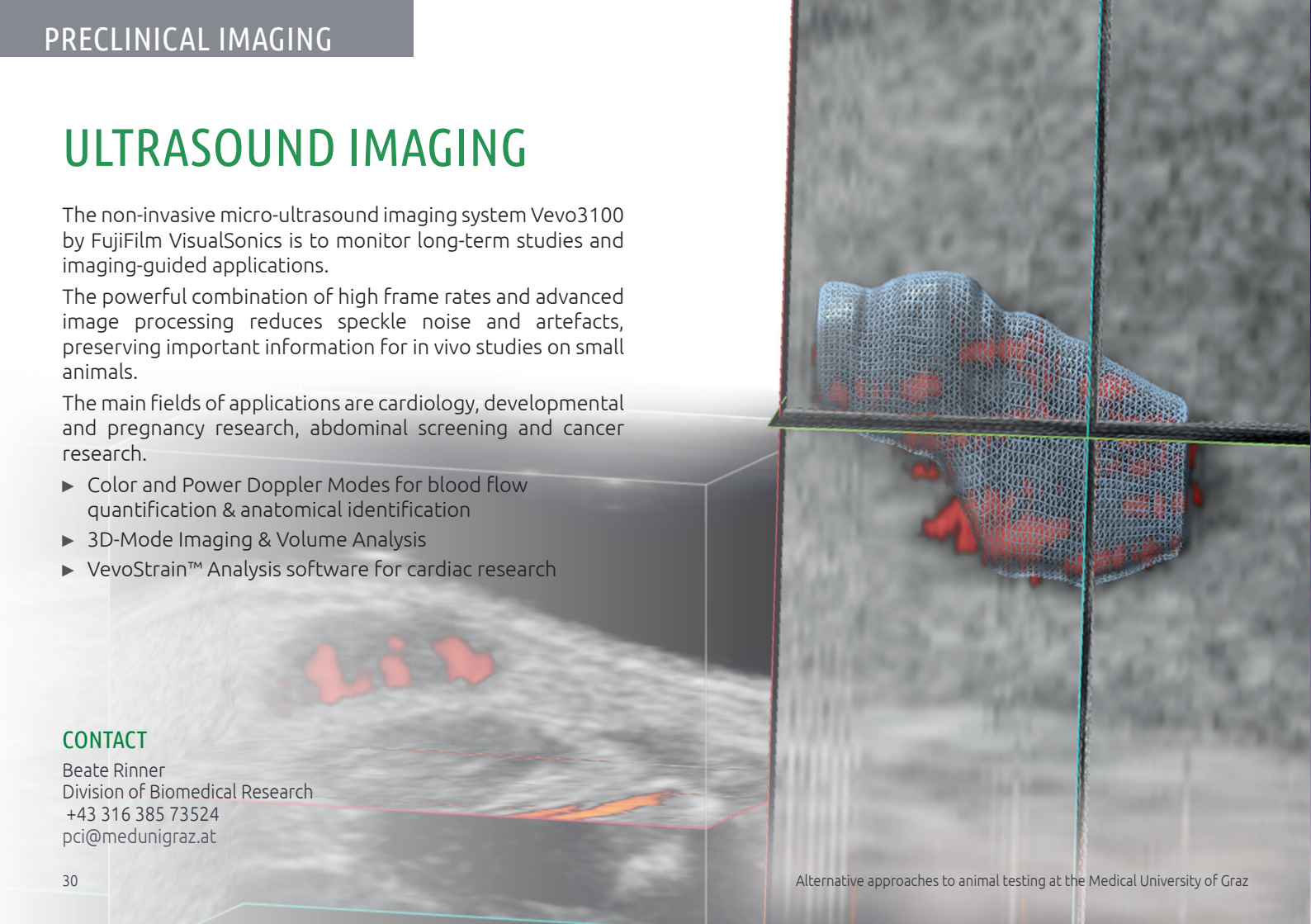
ULTRASOUND IMAGING

The non-invasive micro-ultrasound imaging system Vevo3100 by FujiFilm VisualSonics is to monitor long-term studies and imaging-guided applications.

The powerful combination of high frame rates and advanced image processing reduces speckle noise and artefacts, preserving important information for in vivo studies on small animals.

The main fields of applications are cardiology, developmental and pregnancy research, abdominal screening and cancer research.

- Color and Power Doppler Modes for blood flow quantification & anatomical identification
- 3D-Mode Imaging & Volume Analysis
- VevoStrain™ Analysis software for cardiac research



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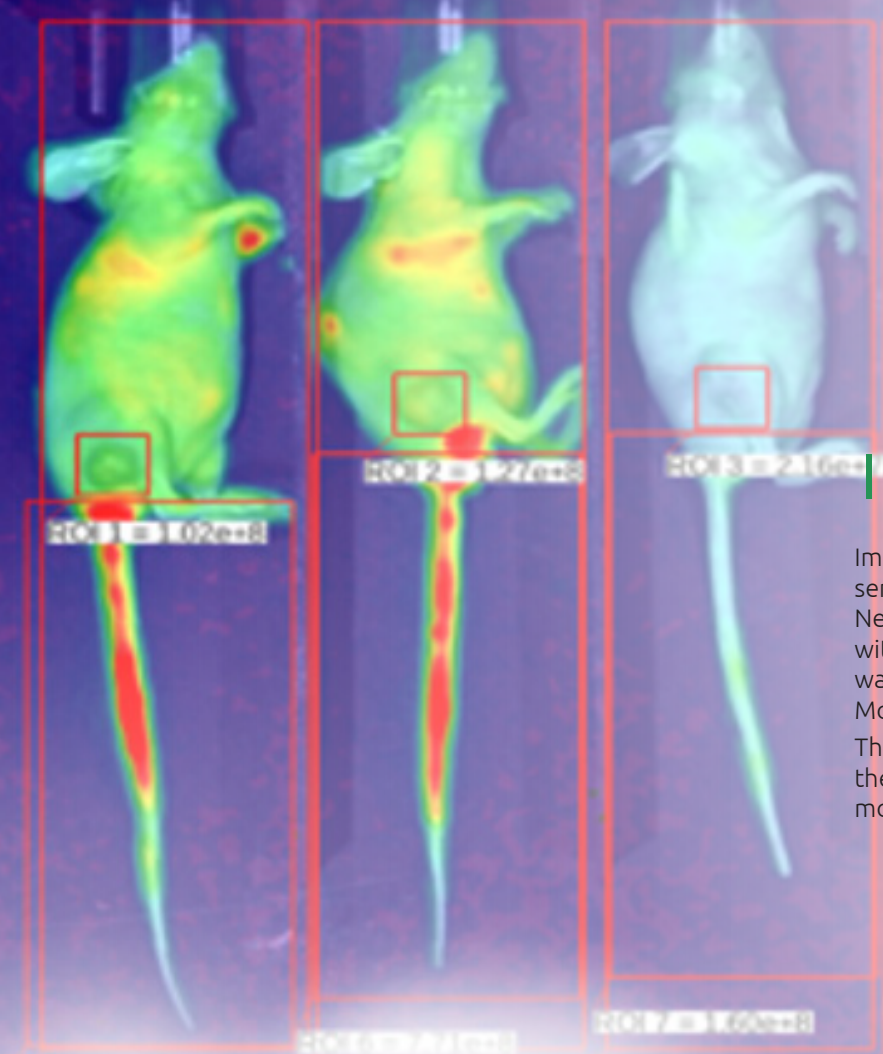
Alternative approaches to animal testing at the Medical University of Graz



IN-VIVO OPTICAL IMAGING

Imaging, Lago OncoMed Solution) provides unrivaled sensitivity for bioluminescence and fluorescence (including Near-infrared) in vivo imaging. The instrument is equipped with LED based illumination, a -90°C cooled Camera, 14 wavelengths/20 filters (no need to filter change), and a 10 Mouse capacity.

The optical imaging system studies disease progression, therapy response, and cell migration in small animal models.

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MAGNETIC RESONANCE IMAGING

MRI is able to enhance the informative value of all *in vivo* studies in addition to being non-invasive, allowing for multiple *in vivo* readouts during a single imaging session and following therapeutic effects over time in the same animal (each animal serves as its own control).

In close cooperation with the Medical University of Graz the Core Facility for Preclinical Imaging operates a 7 Tesla small animal MRI system at the Institute of Molecular Biosciences, University of Graz.

The 7T system is optimized for the use on mice and rats and *in vivo* MRI protocols cover:

- Cardiovascular imaging
- Quantitative blood flow measurements, perfusion measurements
- Volumetric/quantitative fat imaging
- 1H spectroscopy of brain metabolites
- Water diffusion and diffusion tensor imaging
- Tumor imaging and cancer staging
- Cell tracking

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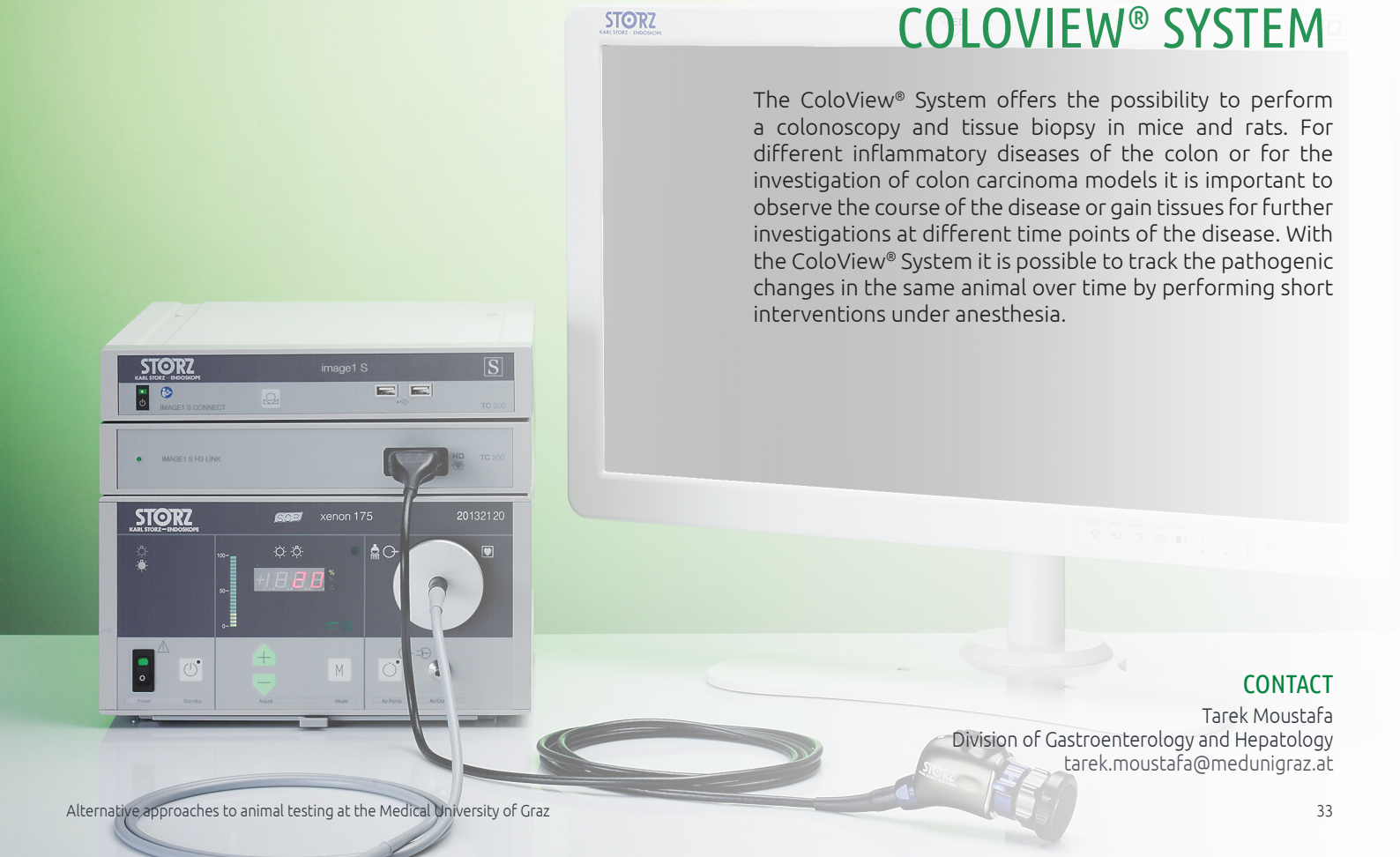
COLOVIEW® SYSTEM

The ColoView® System offers the possibility to perform a colonoscopy and tissue biopsy in mice and rats. For different inflammatory diseases of the colon or for the investigation of colon carcinoma models it is important to observe the course of the disease or gain tissues for further investigations at different time points of the disease. With the ColoView® System it is possible to track the pathogenic changes in the same animal over time by performing short interventions under anesthesia.



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REPREFRED SOCIETY & AUSTRIAN 3R CENTER

The association „Gesellschaft zur Förderung von alternativen Biomodellen" (The RepRefRed Society) was founded by researchers of the Medical Universities of Graz and the with the support of researchers from de Medical Universities of Innsbruck and Vienna the society aims to promote the implementation of the 3Rs. Together with the Austrian 3R Center the association functions as a network for knowledge transfer among scientists and thus improve animal experiment protocols and enforces the reduction and refinement of these experiments by providing different 3R methods.


There are many Ways of Going Forward,
but only one Way of Standing Still.
Franklin D. Roosevelt

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