Division of Surgical Research

Annual Report 2012

Department of Surgery
University Hospital Zurich
Switzerland

University of Zurich[UZH]

UniversityHospital Zurich

Content

Preface

Dear Colleagues

Prof. Dr.
Gregor Zünd, MD
Head Division of
Surgical Research

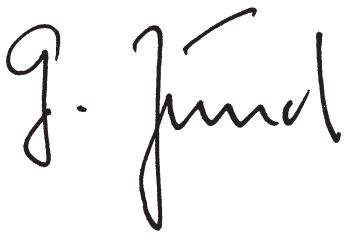
It is my privilege to present the Annual Report 2012 of the Division of Surgical Research at the Department of Surgery, University Hospital Zurich.

The major investments of laboratory equipment made in the past year include the purchase of two experimental monitoring systems and a defibrillator for the large animal OP, an ultra-low temperature freezer, a CO_2 incubator and a GELCOUNT cell colony counter for the regenerative medicine center. Furthermore, we had to replace defect equipment and acquired three biological safety cabinets and a fully motorized rotary microtome.
In the large animal division, we inaugurated in June 2012 a new future-oriented high-technology unity: The hybrid operating room.

For teaching activities, several wet lab events for surgeons and microsurgery classes for surgical residents were offered. The weekly lectures held by the Division of Surgical Research at the University Hospital Zurich were regularly attended by the members of our Division and other scientists representing an integrative part of the academic curriculum within the University, University Hospital and the Swiss Federal Institute of Technology.

It is my great pleasure to thank all members within our Division as well as our research partners of the University, University Hospital and the Swiss Federal Institute of Technology for last year's excellent contributions and fruitful collaborations.

Yours sincerely



Prof. Dr. Gregor Zünd, MD
Head Division of Surgical Research

1. Organisation

1.1 Position of the Division of Surgical Research within the Department of Surgery

Prof. Dr.
Pierre-Alain Clavien,
MD, PhD
Director Clinic of
Visceral & Transpl.
Surgery

Prof. Dr.
Hans-Peter Simmen,
MD
Director Clinic of
Trauma Surgery

Prof. Dr.
Walter Weder,
MD
Director Clinic of
Thoracic Surgery

Prof. Dr.
Volkmar Falk,
MD
Director Clinic of
Cardiovascular Surgery

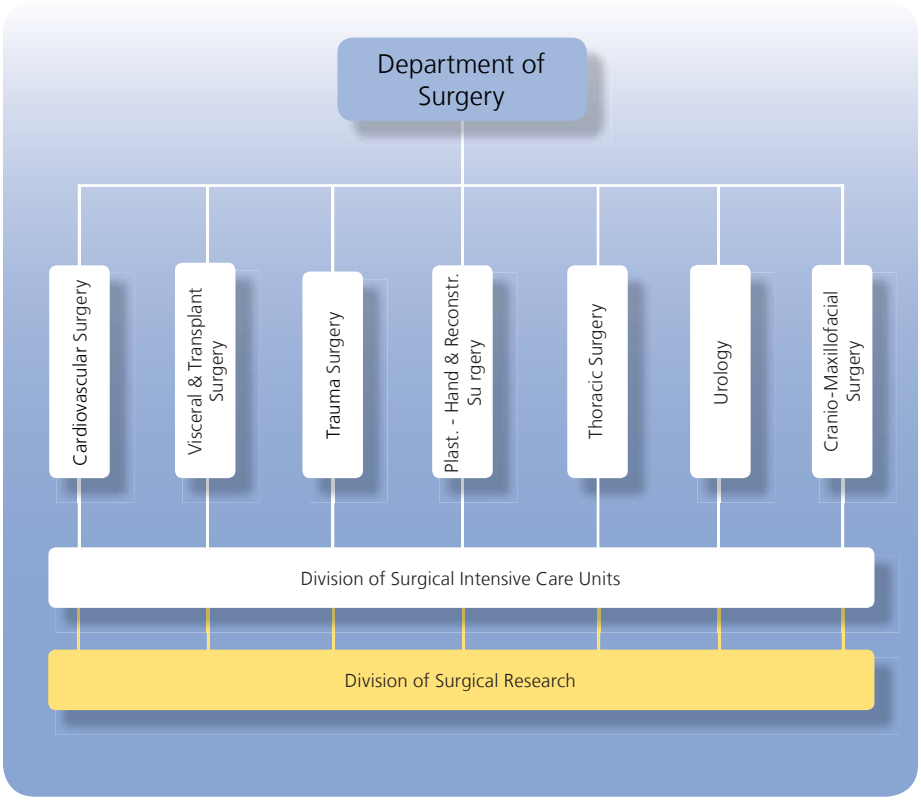
Prof. Dr.
Pietro Giovanoli,
MD
Director Clinic of
Plastic - Hand &
Reconstr. Surgery

Prof. Dr.
Tullio Sulser,
MD
Director Clinic of
Urology

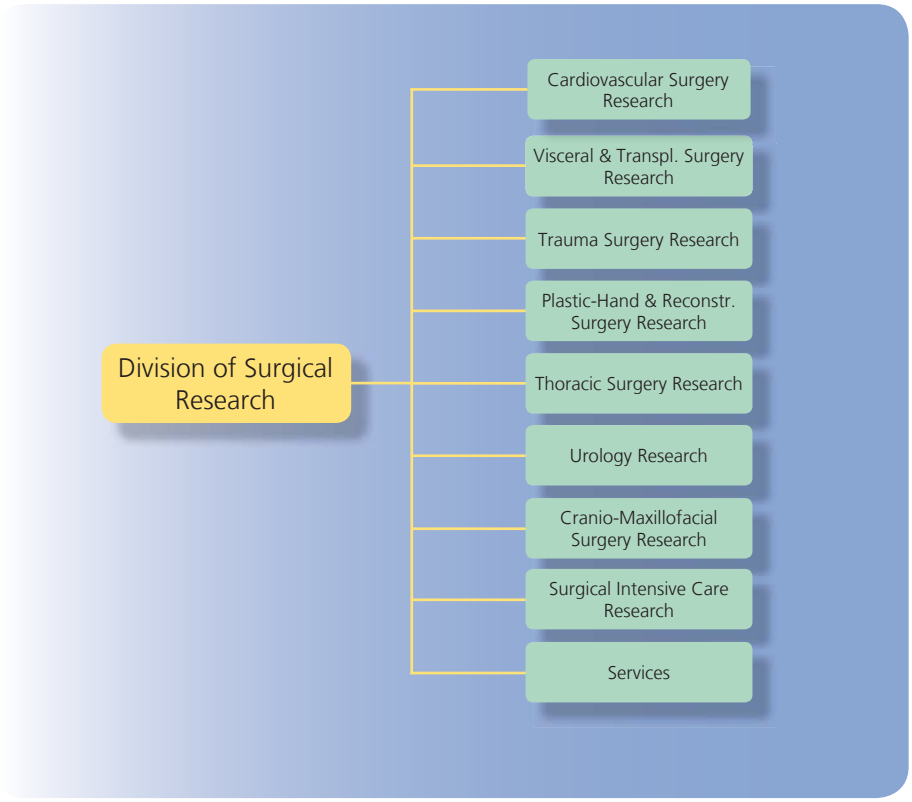
Prof. Dr. Dr. dent.
Klaus W. Grätz, MD
Director Clinic of
Cranio-Maxillofacial
Surgery

Prof. Dr.
John Stover,
MD
Head of Intensive
Care Unit

Prof. Dr.
Gregor Zünd,
MD
Head Division of
Surgical Research



1.2 Structural Organisation of the Division of Surgical Research



Prof. Dr.
Gregor Zünd, MD
Head Division of Surgical Research

Prof. Dr.
Rolf Graf, PhD
Co-Head Division of Surgical Research

Susanne Frehner
Administration
Division of Surgical Research

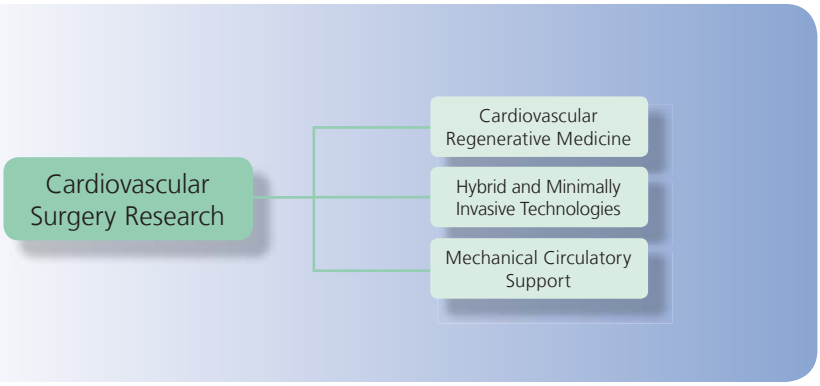
2. Research and Development

2.1 Cardiovascular Surgery Research

Prof. Dr.
Simon Philipp
Hoerstrup,
MD, PhD

Prof. Dr.
Volkmar Falk,
MD, Director

Prof. Dr.
Jürg
Grünenfelder,
MD



Prof. Dr.
Mario Lachat,
MD

Dr.-Ing.
Michael Gessat,

PD Dr.
Maximilian
Emmert,
MD

Dr.
Chad Brokopp,
PhD

Dr.
Benedikt Weber,
MD, PhD

PD Dr.
Markus Wilhelm,
MD

PD Dr.
Stefan Jacobs,
MD

Dr.
André Plass,
MD

PD Dr.
Christian
Schmidt,
MD, PhD

Dr.
Simon
Sündermann,
MD

Dr.
Laura Frese,
PhD

Dr.
Herman Tolboom,
MD

PD Dr.
Dieter Mayer,
MD

Dr.
Zoran Rancic,
MD

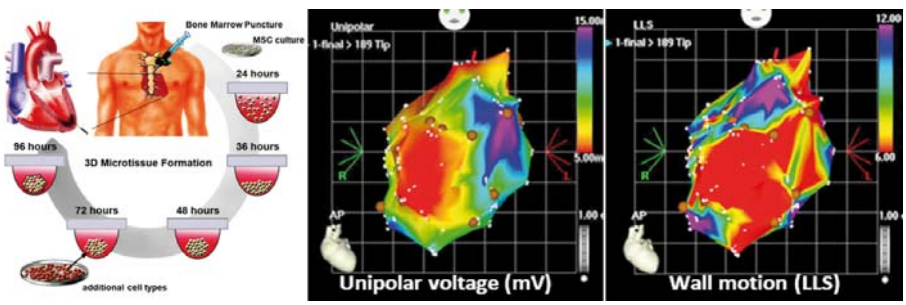
Dr.
Christoph Starck,
MD

2.1.1 Cardiovascular Regenerative Medicine

2.1.1.1 Stem Cells and Microtisssue Technology

M. Emmert, S. P. Hoerstrup

The concept of regenerative medicine has been repeatedly suggested to be an innovative approach to regenerate the diseased myocardium. However, while numerous experimental and preclinical studies using various types of stem and progenitor cells have shown promising results, the outcomes of first clinical pilot trials have only shown marginal effects with regards to the improvement of cardiac performance. Current meta-analyses demonstrate a mean of only 3% improvement of cardiac function. The reasons for the ineffective translation into the clinical setting are related to many unanswered key questions comprising the ideal cell type, the mode of delivery, the optimal timing, the application format (single cells versus 3D concepts), the definition of effective study endpoints as well as the development of imaging techniques to track and monitor cell therapy.



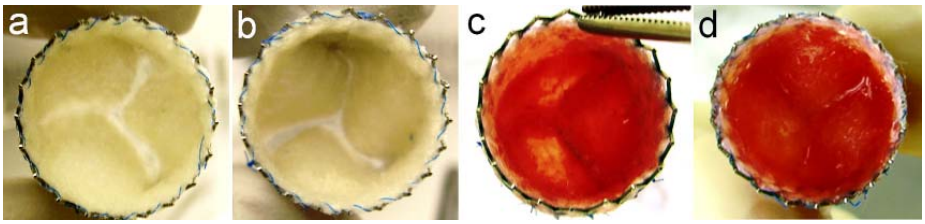
Human mesenchymal stem cell based 3D microtissue generation for advanced myocardial cell therapy (Emmert & Hoerstrup, unpublished concept figure) and intramyocardial injections (red dots) of human mesenchymal stem into the infarcted anterior wall after NOGA Catheter based three dimensional mapping of the left ventricle (red colour; left column) and the corresponding wall motion disruption (red colour; right column).

2.1.1.2 Cardiovascular Tissue Engineering

B. Weber

The strategy of cardiovascular tissue engineering

The main focus of cardiovascular tissue engineering is the development and *in vitro* generation of living tissues for cardiovascular surgery including tissue engineered blood vessels, heart valves as well as patches. Currently utilized heart valve and blood vessel prostheses carry disadvantages for the patients mainly because non-living, artificial devices are inserted into the human organism. Tissue engineering enables the *in vitro* production of autologous, living and functional replacements with the capacity of regeneration and growth - the latter being of particular importance for pediatric application as an alternative to state of the art artificial replacements.



In situ *tissue engineered heart valve based on BMNCs. Valve shaped PGA-P4HB scaffolds (A–B) are seeded with autologous BMNCs (C–D) prior to minimally invasive* in vivo *implantation. (Weber et al., Eur Heart J. 2011 Nov;32(22):2830-40.)*

2.1.1.3 Disease Modeling

J. Robert, B. Weber

The pathogenesis of atherosclerosis involves dysfunctions of vascular endothelial cells and smooth muscle cells as well as blood borne inflammatory cells such as monocyte-derived macrophages. *In vitro* experiments towards a better understanding of these dysfunctions are typically performed in two-dimensional cell culture systems. However, these models lack both the three-dimensional structure and the physiological pulsatile flow conditions of native arteries. We here describe the development and initial characterization of an tissue engineered artery equivalent, which is is composed of human primary endothelial and smooth muscle cells and is exposed to flow *in vitro*. Histological analyses showed a dense tissue formation composed of a tight monolayer of endothelial cells supported by a basement membrane and multiple smooth muscle cell layers. Both low (LDL) and high density lipoproteins (HDL) perfused through the artery equivalent were recovered both within endothelial cells and in the sub-endothelial intima. After activation of the endothelium with either tumor necrosis factor alpha (TNFα) or LDL, monocytes circulated through the model were found to adhere to the activated endothelium and to transmigrate into the intima. In conclusion, the described tissue engineered human artery equivalent model represents a significant step towards a relevant *in vitro* platform for the systematic assessment of pathogenic processes in atherosclerosis.

Thomas Baumgartner
Study Coordination, Administration

Dr. Ajit Sankar Mallik, MD, PhD

Dr. Lyubov Chaykovska, MD

Dr. Steffen Zeisberger, PhD

Simon Schams, M.Sc.

Jérôme Robert, PhD Student

Raoul Hopf, Dipl. Ing.

Pascal Heye, M.Sc.

Boris Jenni, M.Sc.

Francesca Papadopulos, M.Sc.

Yves Wyss, cand.med.

Christoph Russ, Dipl. Ing.

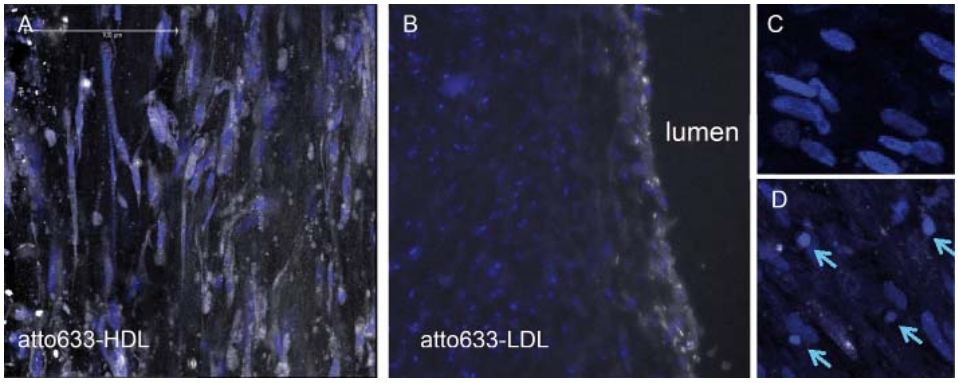
Christine Lohmann, Lab. Manager

Debora Kehl, M.Sc.

Agnieska Ksiazek, Student vet.med.

Ursula Steckholzer, Lab. Technician

Petra Wolint, Lab. Technician

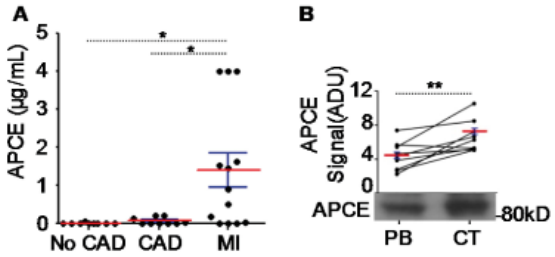


A: localization of HDL in the engineered artery. Confocal image of the localization of HDL (white) in the engineering artery. HDL demonstrated a vesicular localization as well as a diffuse localization. B: localization of LDL (white) in the engineered artery. The LDL is localized in the endothelium and in subendothelial space of the engineered artery. C&D: monocyte adhesion in non activated endothelium (C) or after activation of the endothelial cells with TNFα (D). After activation the number of adherent monocytes is enhanced (green arrows).

2.1.1.4. Novel Targets for Infarction Prevention Strategies

C. Brokopp

Our group is engaged in research to identify novel diagnostic and therapeutic targets useful for myocardial infarction prevention. To this end, we have found evidence that a circulating serine protease, Anti-plasmin Cleaving Enzyme (APCE), is increased patients suffering from myocardial infarction. APCE is also shown to be induced by inflammation and contribute to thrombosis in animal models, indicating that APCE-inhibition may also hold potential as a therapeutic target for myocardial infarction prevention. Ongoing work seeks to identify the prognostic value of circulating APCE in predicting myocardial infarctions in addition to evaluating ACPE-inhibiting therapies for heart attack prevention.



A. Plasma APCE levels are enhanced in patients suffering myocardial infarction (MI), versus patients with stable coronary artery disease (CAD), or no coronary artery disease (No CAD).
B. ACPE levels are enhanced in obstructive acute coronary thrombi (CT) vs. peripheral blood (PB) in patients suffering from an ST elevation myocardial infarction.

Collaborations:

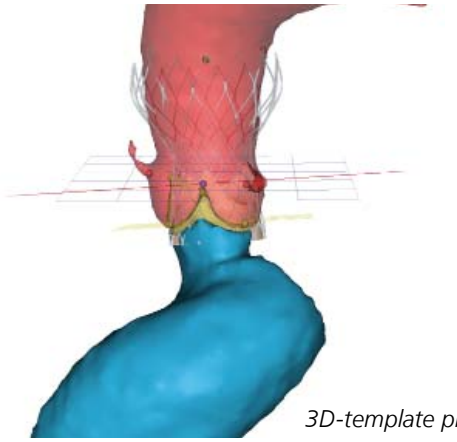
- Department of Biomedical Engineering, Technical University Eindhoven, The Netherlands
- Center for Integrative Human Physiology, University of Zurich, Switzerland
- Department of Materials, Federal Institute of Technology, Zurich, Switzerland
- Department of Biochemistry, University Zurich, Switzerland
- Department of Mathematics, Federal Institute of Technology, Zurich, Switzerland
- Department of Computational Science, Federal Institute of Technology, Zurich, Switzerland
- Department of Veterinary Surgery, MSRU Vetclinics, University Zurich, Switzerland
- Department of Cardiac Surgery, Children's Hospital, Harvard Medical School, Boston, MA, USA
- Department of Pathology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA
- Massachusetts Institute of Technology (MIT), Cambridge, MA, USA
- Laboratory for Tissue Engineering, German Heart Centre, Berlin, Germany
- Department of Cardiology, Medical University of Vienna, Austria
- Institute of Nuclear Medicine, University of Debrecen, Hungary
- Institute of Chemistry and Applied Biosciences, Federal Institute of Technology Zurich, Switzerland
- Institute of Anatomie, University of Bern, Switzerland
- Human Genetics Laboratory, Genetica AG, Zurich, Switzerland
- Departments of Pathology, Neurosurgery, Cardiology, and Laboratory for Transplantation Immunology, University Hospital, Zurich, Switzerland
- Randall Division of Cell and Molecular Biophysics, King's College London, UK
- Embryonic Stem Cell Laboratory, Fraunhofer Institute for Biomedical Engineering IBMT, St. Ingbert, Germany
- Department of Pathology and Immunology, Geneva University, Switzerland
- Experimental Cardiology Unit, Department of Medicine, University of Lausanne Medical School, Switzerland

2.1.2 Hybrid and Minimally Invasive Technologies

2.1.2.1. The Advanced Role of Imaging in Transcatheter Cardiac Valve Treatment

S. Sündermann

Interventional, catheter based treatment of heart valve disease has become a widely accepted alternative option for the treatment of aortic and mitral valve pathologies in patients at high risk for surgery. Together with engineers at the ETH Zürich and Philips Healthcare in Best (Netherlands) we are exploring the capabilities offered by the new infrastructure and software available in in a Hybrid-OR as essential parts of those procedures. This contains software tools for intervention planning for transcather aortic valve implantation (TAVI) and for navigation during MitraClip procedures. Also an own software tool was developed and validated for preoperative TAVI planning.

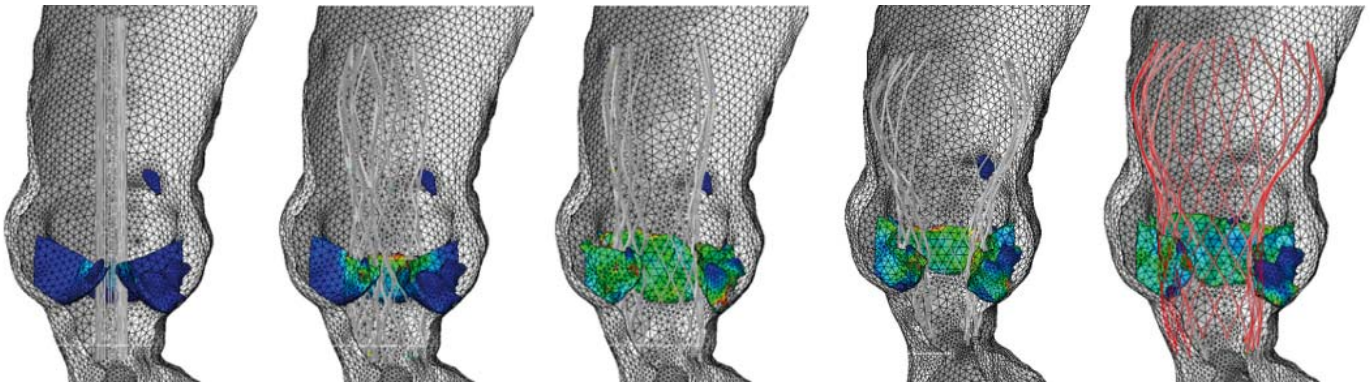


3D-template planning tool

2.1.2.2 Computational Models in Cardiac Surgery

M. Gessat

Computational models provide phyisicians with a so called "in-silico" representation of a patient which integrates all kinds of background knowledge about anatomy, physiology, biophysics, biochemistry and other sciences with data collected from a particular patient. Such models are used, e.g., to predict the outcome of a given treatment strategy, for instance the transcatheter implantation of a stented valve implant in a patient with severe aortic stenosis. The Hybrid Laboratory for Cardiovascular Technologies runs several collaborative projects with the ETH Zürich, aiming at developing and clinically applying such models for cardiovascular surgery.



Prospective, patient specific simulation of TAVI stent deployment with elastic leaflets and rigid aorta.

Collaborations:

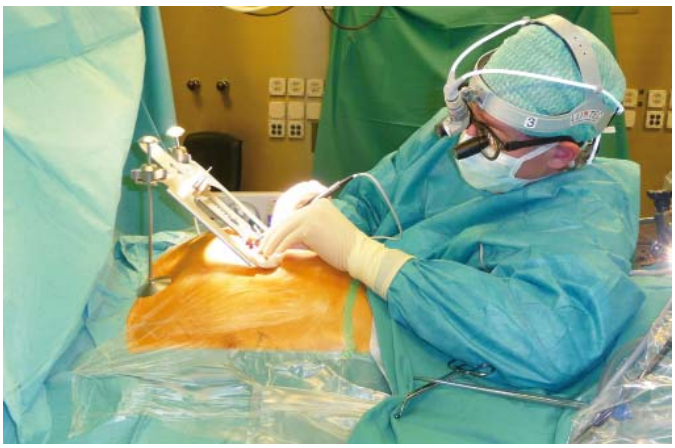
- Philips Healthcare (Best, Netherlands)
- Swiss Federal Institute of Technology (ETH) Zurich, Computer Vision Laboratory (Zurich, Switzerland)
- Swiss Federal Institute of Technology (ETH) Zurich, Centre for Mechanics (Zurich, Switzerland)
- University of Stanford, Living Matter Lab (Stanford, USA)
- University of Pavia, Structural Mechanics Department (Pavia, Italy)
- Hochschule Karlsruhe, Fakultät für Informatik (Karlsruhe, Germany)

2.1.2.3 Hybrid revascularization

S. Sündermann

Hybrid coronary artery revascularization has gained renewed attention. The hybrid approach seeks to combine the advantages of PCI and minimally invasive CABG, providing the benefits of proven long-term patency of a LIMA-to-LAD graft.

In 2012 revascularization of the left anterior descending artery was performed in 30 patients by minimally invasive direct coronary artery bypass grafting (MIDCAB) followed by Percutaneous Coronary Intervention (PCI) of vessels other than the left anterior descending artery in a single procedure in our surgical hybrid suite with good results. Angiography confirmed patency of all LIMA grafts. Kaplan-Meier survival was 100% at 1 year.



Left lateral small thoracotomy

2.1.2.4 New minimally invasive mitral valve procedures

S. Sündermann

Mitral valve (MV) regurgitation (MR) is the second most common heart valve disease. Besides the gold standard therapy, surgical MV repair, new devices have been developed for repair of the MV. We did studies with a beating-heart adjustable mitral valve ring in a human feasibility and post-marketing surveillance study in humans (11 rings have been implanted successfully in 2011/2012 with good follow-up results), with a transcatheter mitral valve annuloplasty device in an acute animal trial (6 animals have been implanted so far) and with a personalized, patient specific mitral valve ring that was modeled from CT images and afterwards implanted successfully in an animal model.

Figure 1: The Valtech Cardinal adjustable ring (left side) with adjustment handle (right side) and connection line (middle).

2.1.3 Transplantation and Mechanical Circulatory Support

2.1.3.1 Transplantation Research

H. Tolboom

A severe donor organ shortage leads to the death of a substantial number of patients listed for cardiac transplantation. Donors hearts are currently almost exclusively obtained from a very limited pool of brain-dead heart-beating donors. The use of hearts from non-heart beating donors (NHBD) could significantly expand the donor organ pool. Due to concerns about their viability, these ischemic hearts are usually not used for transplantation. Our current research focuses on assessing whether the negative impact of NHBD donation can be reversed with ex-vivo machine perfusion, enabling safe transplantation of NHBD hearts.

2.1.3.2 Mechanical Circulatory Support

Ch. Starck, A. Plass, M. Wilhelm

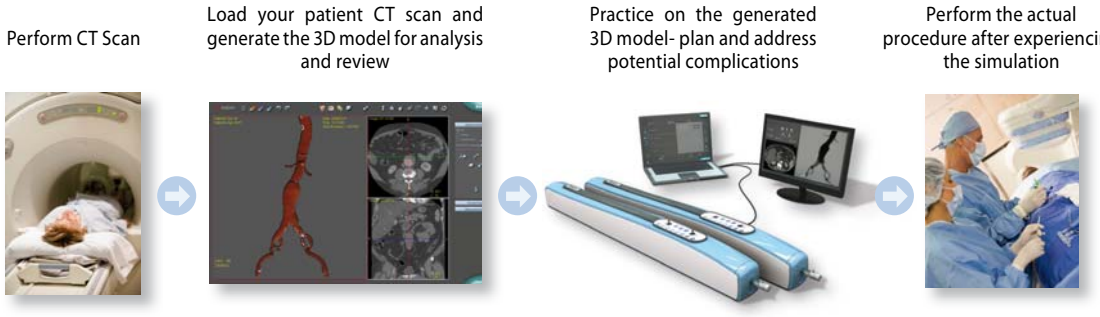
Optimization of critical components of existing assist devices and development of innovative concepts of circulatory support systems are the main goals in this research field. Therefore a cooperation between the Clinic of Cardiovascular Surgery and several research groups of the Swiss Federal Institute of Technology Zurich (ETH) was established. The focus of the different projects is on biocompatibility, physiological adaptivity, transcutaneous energy transfer and new device concepts.

2.1.4 Vascular Surgery

2.1.4.1 Patient-specific virtual reality rehearsal prior to EVAR: Influence on technical and nontechnical operative performance. Randomized Controlled Trial

M. Lachat, Z. Rancic, L. Chaykowska

Vascular disease is a growing problem in aging western populations. Treatment of vascular lesions develops from open surgical approaches to endovascular treatments, which requires a different skills, including work in a three-dimensional field while viewing a two dimensional image, deal with reduced tactile feedback and increased need for hand-eye coordination. Virtual reality simulation of endovascular techniques was established for teaching and practicing. Moreover, patient-specific procedure rehearsal may now be simulated using real images. Patient-specific rehearsal of carotid artery stenting was already proven to improve the outcomes of a real procedure. The patient-specific rehearsal software is available for endovascular repair of infrarenal aortic aneurysms (EVAR), which may improve device selection, increase technical performance of surgeon/radiologist and communication within the endovascular team. However, further research is needed to evaluate if this new technology significantly improves procedure quality.



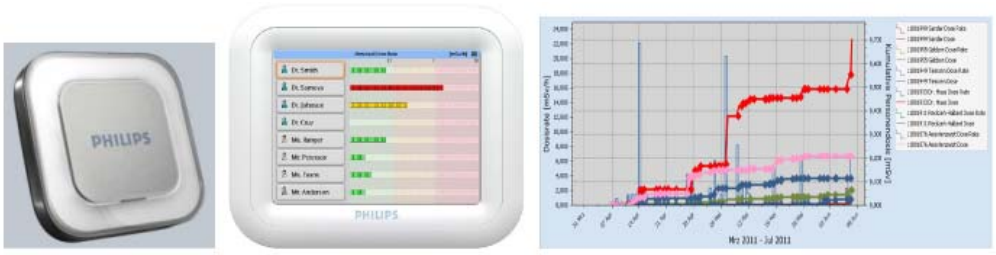
Collaborations:

- Prof. Dr. Isabelle Van Herzeele, Ghent University Hospital (Gent, Belgium) and Sint - Maarten Hospital (Duffel, Belgium)

2.1.4.2 Evaluation of the impact of DoseAware in a clinical setting

M. Lachat, L. Chaykowska

Endovascular abdominal aneurysm repair (EVAR) is the procedures with the highest radiation exposure dose (76-119 mSv). Exposure of medical staff to radiation is unavoidable, due to the high radiation burden of the imaging procedure, but it may and should be reduced. Although radiation dosimetry (TLD) badges are mandatory, the staff generally pays little heed to the monthly or cumulative reports of radiation exposure. Thus radiological awareness among physicians should be optimized. The DoseAware system allows a real time display of the actual X-ray dose a staff member receives. In addition the staff member can view its personal accumulated dose directly after the procedure, at the end of the day or for any specified time range. It is expected that the shortened feedback loop and increased awareness will have direct effect on behavior of the staff and reduce radiation dose and exposition during procedures.



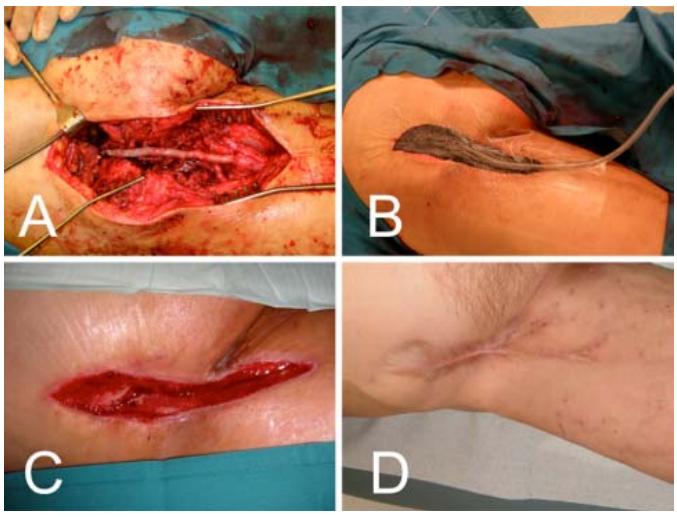
Collaborations:

- Philips Healthcare (Netherland)

2.1.4.3 Vascular graft infections and negative pressure wound therapy (NPWT)

D. Mayer

VASGRA is a project funded by the Swiss National Foundation (SNF) that aims to elucidate the epidemiology, best treatment options, imaging modalities of vascular graft infections and the impact of negative pressure wound therapy on morbidity and mortality. A prospective observational cohort database of all patients will be created who receive a vascular graft (peripheral or aortic, prosthetic or vein) at the University hospital of Zurich (VASGRA Cohort A). Patients with a vascular graft infection will be included in VASGRA Cohort B and followed up using a flow chart with a focus on the course of this infectious complication. The expected value of the VASGRA project is to provide an important contribution to the research field of vascular graft infections, based on the large sample size, longitudinal design and by unifying clinical and epidemiological science. With the gained knowledge, it should be possible to develop guidelines regarding best diagnostic modalities and treatment options in case of vascular graft infections.

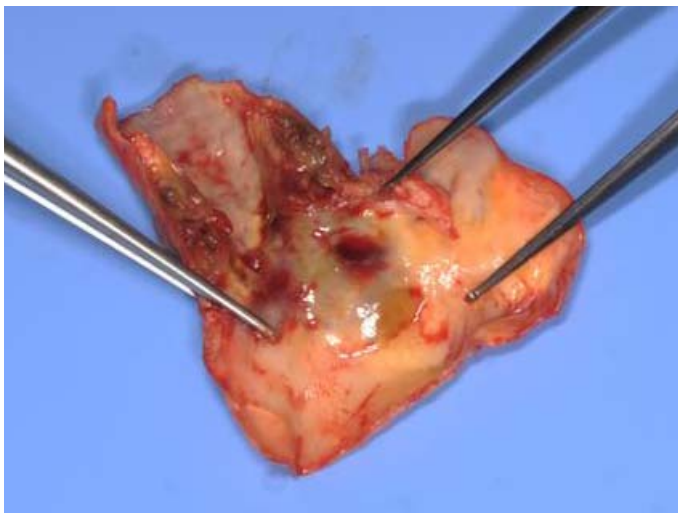


2.1.4.4 Identification of human vulnerable atherosclerotic plaques with Positron-Emissions Tomography (PET)

Z. Rancic

Through the PET diagnostics vulnerable plaques can be selectively identified and initiated a pharmacological therapy or surgical intervention. The goal of this project is the identification and validation of overexpressed structures in vulnerable atherosclerotic plaques using biochemical methods and the evaluation of PET ligands for these targets.

At our department, Clinic for Cardiovascular Surgery we perform carotid endarterectomy mostly for symptomatic disease. Using a technique of advanced carotid bifurcation plasty is possible to store separately endarterectomized plaques obtained from common, internal and external carotid artery. After plaque removal we assess the stability of the plaque. Then the plaques are stored in a biobank at the ETH Zurich. The tissues are used for the identification and evaluation of novel targets for the selective detection of vulnerable plaques. The techniques are in use are: the reverse transcriptase polymerase chain reaction (RT-PCR), autoradiography, histological staining, Western blotting, and immunohistochemistry. After a thorough evaluation target PET ligands are developed and investigated in *in vitro* and *in vivo* experiments. The experiments in cell and animal models allow conclusions on biochemical and pharmacokinetic characteristics of the examined diagnostic radiopharmaceuticals.



Vulnerable atherosclerotic plaque removed from the carotid arteries during carotid endarterectomy. The tissues are used for the identification and evaluation of novel targets for the selective detection of vulnerable plaques.

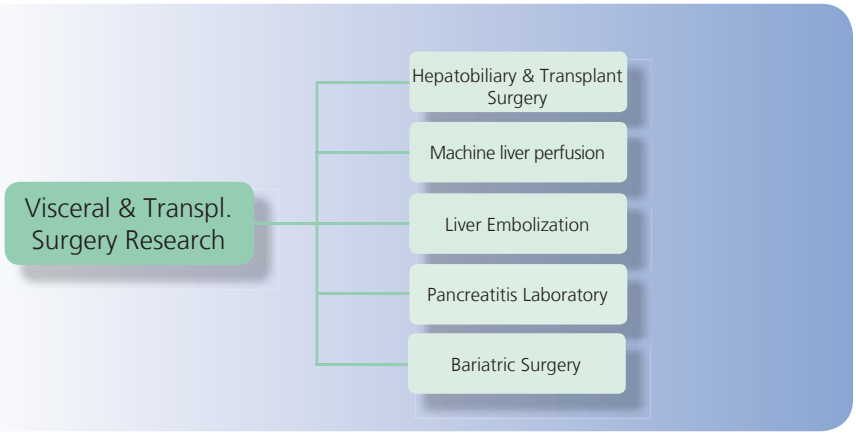
Collaborations:

- Swiss Federal Institute of Technology (ETH) Zurich, Institute of Pharmaceutical Sciences
- Center for Radiopharmaceutical Sciences, Team Leader: Prof. R. Schibli, PD Dr. S. Krämer, Dr. A. Müller, R. Meletta

2.2 Visceral & Transplant Surgery Research

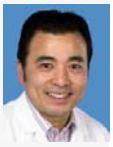

Prof. Dr.
Rolf Graf,
PhD


Prof. Dr.
Pierre.Alain
Clavien,
MD, PhD, Director




Prof. Dr.
Philipp
Dutkowski,
MD


Prof. Dr.
Mickael Lesurtel,
MD, PhD


PD Dr.
Yinghua Tian,
MD

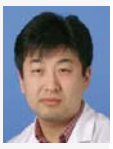

Dr.
Sabrina Sonda,
PhD


Dr.
Marco Bueter,
MD, PhD


Dr.
Bostjan Humar,
PhD


Dr.
Ksenija
Slankamenac,
MD


Dr.
Kuno Lehmann,
MD


Dr.
Jae Hwi Jang
PhD, Postdoctoral
Fellow


Dr.
Andrea Schlegel,
MD


Dr.
Christoph
Tschuor,
MD


Dr.
Perparim Limani,
MD


Dr.
Michael Linecker,
MD


Dr.
Andrea Vuck,
MD


Dr.
Gitta Maria
Seleznik,
M.Sc.

2.2.1 Hepatobiliary & Transplant Surgery

miR-21 and the control of liver regeneration in the Small-for-Size Syndrome

E. Kachaylo, Ch. Tschuor, M. Linecker, J.-H. Jang, P. Limani, P. Ramadori, M. Foti, R. Graf, B. Humar, P.-A. Clavien

Small-for-Size Syndrome (SFSS) puts patients at risk of post-operative death and can develop (**i**) following transplantation of critically small grafts or (**ii**) following extended hepatectomy leaving marginal remnants. We have identified delayed liver regeneration as a cause of SFSS, however the underlying molecular events remain ill-understood. miR-21, an oncogenic microRNA associated with proliferative states, is induced after standard (70%) hepatectomy, but not after extended (86%) hepatectomy which leads to SFSS in mice. Using miR-21 mimics injected before 86% hepatectomy, we could accelerate liver regeneration and rescue mice from SFSS. To uncover the molecular processes governed by miR-21, we will investigate various mousy models featuring gain or loss of miR-21 function. These experiments will contribute towards a molecular definition of the SFSS and reveal novel targets for a potential intervention. *(Figure 1)*

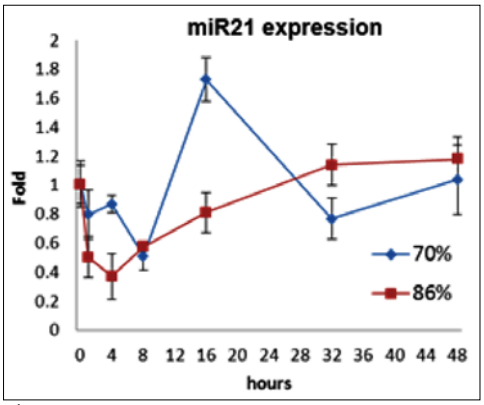


Figure 1

Constitutive androstane receptor agonism in the mitigation of the Small-for-Size Syndrome

Ch. Tschuor, E. Kachaylo, P. Limani, A. Schlegel, J.-H. Jang, D. A. Raptis, E. Melloul, M. Linecker, A. Vuck, Y. Tian, R. Graf, B. Humar, P.-A. Clavien

Activation of the constitutive androstane receptor (Car) through its agonist TCPOBOP (TCP) induces liver growth without the need for resection or injury in rodents. Indeed, TCP also induces liver growth in experimental models of SFSS, overriding the regenerative deficits of marginal liver grafts or remnants. In SFSS models of transplantation and resection, a single dose of TCP was able to rescue 40% of otherwise moribund mice, even when given a few hours after surgery. Current experiments using siRNA technology have identified the cell cycle promoter Foxm1b as a critical component mediating the regenerative effects downstream of TCP-Car. Experiments using a humanised CAR mouse, human SFSS biopsies, and *ex vivo* liver slices together with the human CAR agonist CITCO, will reveal whether CAR agonism may also be a viable strategy to mitigate human SFSS. *(Figure 2)*

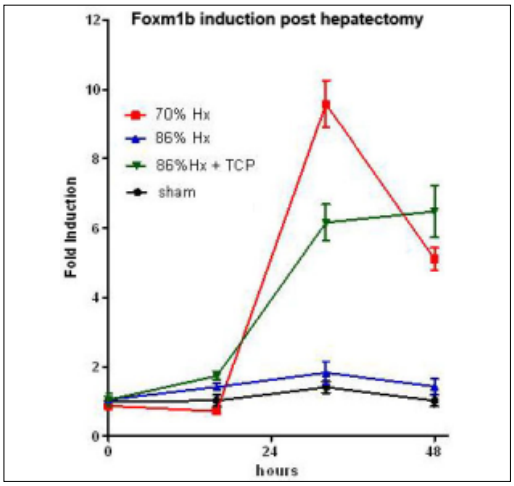


Figure 2

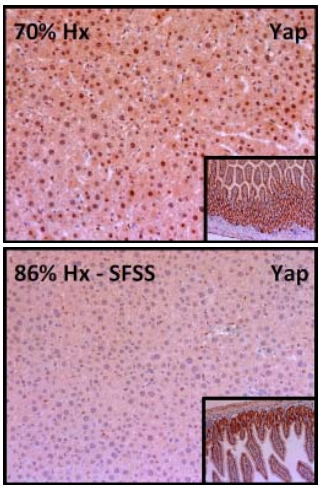


Figure 3

The miR-375-Yap pathway in liver regeneration and the Small-for-Size Syndrome

Ch. Tschuor, E. Kachaylo, A. Schlegel, P. Limani, A. Columbano, J.- H. Jang, D. A. Raptis, E. Melloul, M. Linecker, A. Vuck, Y. Tian, R. Graf, B. Humar, P.-A. Clavien

In our efforts to understand the molecular events that underlie the SFSS, we have observed an overinduction of miR-375 along with concomitant downregulation of its direct target Yap, a repressor of the Hippo pathway which limits organ size. Intriguingly, miR-375 has been reported to be repressed by Car agonism, suggesting Car may induce liver overgrowth by promoting Yap to suspend organ size control. However, whether the miR-375-Yap axis has a crucial function during physiological liver regeneration is not known. To establish a role for this pathway in hepatic regeneration, we will assess the effects of miR-375 mimics/antagomirs and Yap siRNA in models of liver regeneration induced by either resection, extended resection, and/or Car agonism. *(Figure 3)*


Dipl. phil. II Theresia Reding Graf


Dr. Dimitri A. Raptis, MD


Kamile Grabliauskaite, M.Sc.


Enrica Saponara, M.Sc.


Katja Kachaylo M.Sc.


Martha Bain Lab. Technician


Udo Ungethüm Lab. Technician


Nadja Bain M.Sc.


Eleonora Maurizio Lab. Technician

Pten function in liver regeneration

E. Kachaylo, Ch. Tschuor, M. Linecker, J.-H. Jang, P. Limani, P. Ramadori, M. Foti, R. Graf, B. Humar & P.-A. Clavien

Pten, the well-known negative regulator of the growth-related PI3K-Akt-mTOR pathway, has many additional, emerging functions. In liver, Pten deficiency causes lipid accumulation, leading to steatohepatitis and cancer. Given that liver regeneration features both tissue growth and transient hepatic steatosis, Pten regulation might be crucial after hepatectomy. Preliminary data indicates a drop in Pten levels following liver resection. Using hepatocyte-specific inducible Pten knockout mice, we will investigate whether suppression of Pten function is a requirement for liver regeneration and whether Pten serves to orchestrate energy demands and growth pathways during tissue renewal. *(Figure 4)*

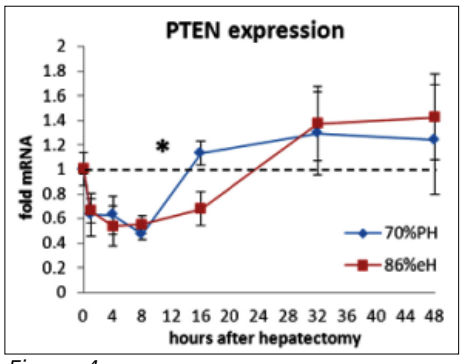


Figure 4

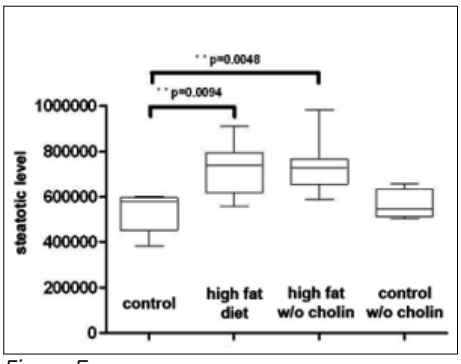


Figure5

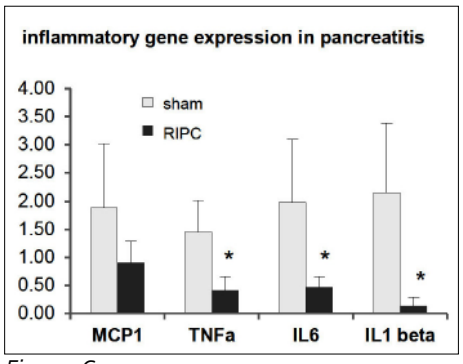


Figure 6

The impact of exercise and ω3-fatty acids on ischemia reperfusion injury in and regeneration of fatty liver

M. Linecker, M. Foti, A.-Ch. Piguet, J.-F. Dufour, E. Kachaylo, P. Limani, Ch. Tschuor, A. Schlegel, A. Vuck, D.A. Raptis, R. Graf, B. Humar, P.-A. Clavien

The current surge in obesity is the likely cause for the increasing number of patients that present with fatty liver (steatosis). Hepatic lipid accumulation increases the risk of liver cancer and hence contributes to an increased demand for liver surgery. However, fatty liver also is exquisitely sensitive towards ischemia reperfusion injury (IRI) and slows liver regeneration, thereby increasing postoperative complications. Exercise and ω3-fatty acids are known to counteract steatosis, but their effects on IRI and regeneration in fatty livers are less clear. We will investigate the potency of daily exercise and ω3-fatty acids in lean mice, mice on a high fat diet, and in genetically obese mice. We will focus on AMPkinase as a potential target common to exercise and ω3-fatty acids, and on Pten, because downregulation of this molecule is a frequent finding in human steatosis. Any intervention that can safely reduce hepatic fat accumulation and mitigate IRI without negatively affecting the regenerative capacity would be most welcome in the clinic. Inasmuchω3-fatty acids can substitute the beneficial effects of exercising is another point of interest, as regular exercise will always remain limited in its therapeutic potential due to matters of compliance. *(Figure 5)*

Remote ischemic preconditioning beyond protection from ischemia reperfusion injury

P. Limani, Ch. Oberkofler, Ch. Tschuor, A. Schlegel, E. Kachaylo, M. Linecker, R. Graf, B. Humar & P.-A. Clavien

We have recently identified a platelet-serotonin-Vegf-Mmp8/Il10 axis that mediates most of the protective effects remote ischemic preconditioning (RIPC) has on ischemia-reperfusion injury (IRI). Given that serotonin can have many beneficial effects such as promoting the regeneration of old liver, RIPC may serve as a safe and physiological way to elevate peripheral serotonin in patients, i.e. without causing unwanted neurological effects that can occur following the injection of serotonin or its analogues. Preliminary data indicates RIPC is able to boost the regeneration of mouse liver and to mitigate acute pancreatitis. We will assess the effects of RIPC on a number of other (patho-) physiological processes, with the aim to promote the use of RIPC beyond its current application for the protection from IRI. *(Figure 6)*

Antihypoxic therapy for colorectal cancer liver metastases

P. Limani, J.-M.- Lehn, A. Schlegel, Ch. Tschuor, E. Kachaylo, J.-H. Jang, R. Graf, B. Humar & P.-A. Clavien

Hypoxia, a key feature of many solid tumors, predicts a bad outcome in colorectal cancer (CRC) liver metastases. Tumors respond to hypoxia by metabolic adaptation, an enhanced inflammatory state, an increased tumorigenic potential, pronounced invasiveness and a neoangiogenic switch. Current targeted therapies for CRC liver metastases such as Avastn specifically block the angiogenic response. However, their impact on overall survival is marginal, likely because anti-angiogenic therapy may promote hypoxic conditions and thereby an aggressive tumor phenotype. Inositol trispyrophosphate (ITPP) is the first non-toxic compound able to efficiently inhibit hypoxia. We have tested ITPP on two syngeneic mouse models of CRC liver metastasis. ITPP prevented several processes typically associated with hypoxia, promoted a mesenchymal-to-epithelial transition, and prolonged survival by 60%. A direct comparison of ITPP plus standard chemotherapy vs. Avastin plus chemotherpy will reveal whether ITPP can prevent the neoangiogenic switch without increasing tumor aggressiveness. *(Figure 7)*

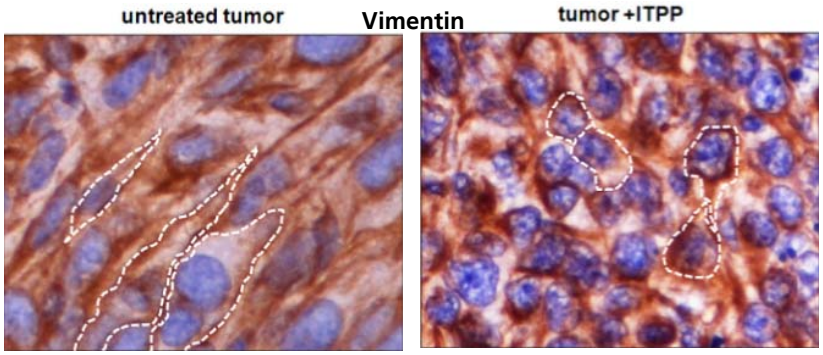


Figure 7

Counteracting ischemia during organ preservation

A. Schlegel, P. Limani, R. Graf, P. Dutkowski, J.-M. Lehn, B. Humar & P.-A. Clavien

The time that elapses between organ harvest and transplantation is a critical period with profound impact on the viability of the graft. In order to improve the outcome of liver transplantations, graft storage prior to implantation needs optimization to minimise tissue injury. Given that graft storage essentially comprises an ischemic period followed by reperfusion, we reasoned that the antihypoxic molecule inositol trispyrophosphate (ITPP) may have beneficial effects on graft quality. A new model of isolated rat liver perfusion (IPRL) involving complete canulation was developed. To mimic the clinical situation, rat livers were harvested 45 minutes after induction of cardiac arrest. For graft preservation, livers were reperfused with an oxygenated solution consisting of full rat blood or isolated erythrocytes and Krebs–Henseleit–Buffer, both with or without ITPP. Antihypoxic treatment resulted in markedly reduced organ injury, increased bile flow and improved perfusion. ITPP-treated grafts used for rat transplantation resulted in a long-term survival of recipients, whereas a significant proportion of rats with untreated grafts died due to biliary cirrhosis. Therefore, ITPP has the potential to improve the outcome of liver transplantation. *(Figure 8)*

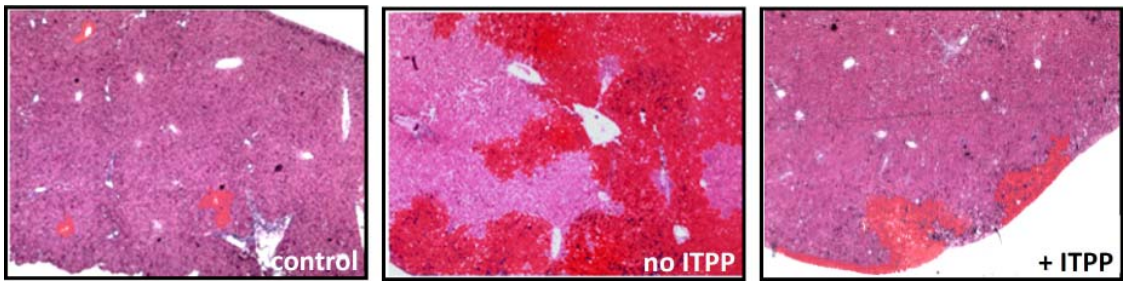
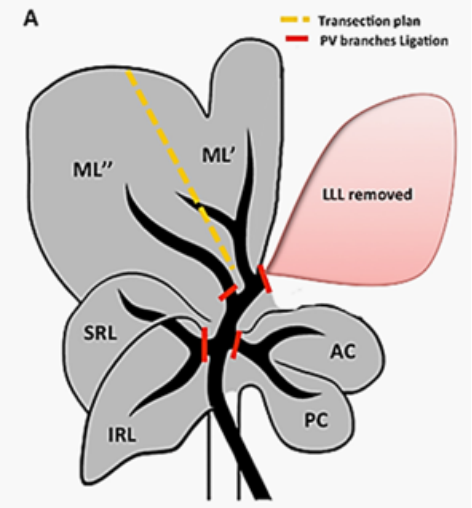


Figure 8

A mouse model of ALPPS

A. Schlegel, E. Melloul, Ch. Tschuor, P. Dutkowski, R. Graf, B. Humar & P.-A. Clavien

ALPPS (for Associating Liver Partition and Portal Vein Ligation for Staged Hepatectomy) is a surgical technique that has newly been introduced in the clinic to enable the resection of large parts of the liver. So far, usual approaches were based on portal vein ligation (PVL) of liver lobes to be removed (i.e. carrying a tumor). PVL induces hypertrophy of the contra-lateral liver lobes, enabling resection of the ligated lobe(s) a few months later even in situations, where the liver remnant would normally be too small to maintain vital function. In ALPPS, PVL is combined with lobe transsection, resulting in an unprecedented volume gain within a week. Therefore, ALPPS allows for a resection of even larger volumes than PVL within a drastically shorter time period. However, ALPPS is not established in the clinic, and its true advantages are a matter of controversial discussion. To de onstrate the utility of ALPPS, we have developed a mouse model of PVL combined with transsection. Indeed, following ALPPS, the liver remnant regains volume within a day, a process that usually takes a week in mice. The data from our model fully supports the boost in the regenerative speed following ALPPS. Using this model, we plan to Investigate the mechanisms that control the speed with which liver can regenerate.



The Comprehensive Complication Index - A Novel Continuous Scale to Measure Surgical Morbidity

K. Slankamenac, R. Graf, J. Barkun, M. A. Puhan, P.-A. Clavien

The quality of surgical procedures has been often measured by the presence of postoperative complications but there is an incomplete reporting of all surgical complications. Furthermore, the severity of complications is often unclear. Alternatively, only the most severe complications are reported. Therefore, we developed a comprehensive complication index (CCI) which summarizes for the first time all postoperative complications and their respective severities in a single number ranging on continuous scale from 0 (none) to 100 (death). Four different validations showed that the CCI has a high validity and better responsiveness than previous reports of postoperative complications. The CCI has the potential to longitudinally measure the morbidity and to serve as a standardized endpoint in surgical trials and other interventional fields of medicine.

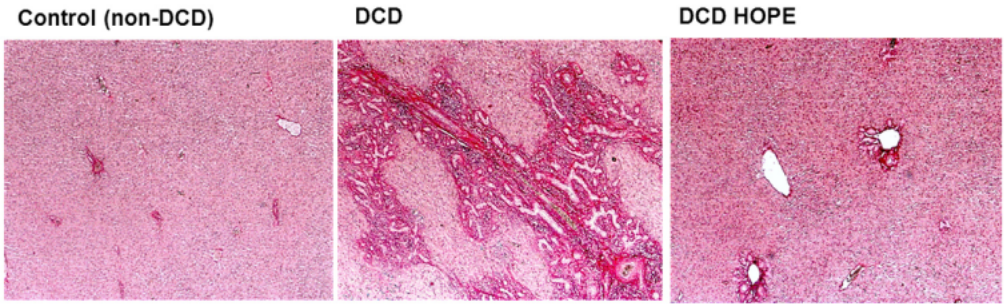
Collaborations/Sponsors:

- Prof. Dr. Adriano Fontana, MD, Experimentelle Immunologie (UniversityHospital Zurich, Switzerland)
- Prof. Dr. Bruno Stieger, PhD, (UniversityHospital Zurich, Switzerland)
- Prof. Dr. Achim Weber, MD, Institut für Pathologie (UniversityHospital Zurich, Switzerland)

2.2.2 Machine Liver Perfusion

A. Schlegel, R. Graf, P.-A. Clavien, P. Dutkowski

In an attempt to reduce the donor organ shortage, transplant centers worldwide accept livers from donation after cardiac death (DCD) donors. Machine perfusion techniques were established to improve these livers. Most studies focus on early graft injury and report on seven day survival, while the effect on biliary injury, remain unclear. In our experiments rat livers were subjected to 30 min warm ischemia, followed by 4hr cold storage and liver transplantation. DCD livers in the HOPE group underwent also passive cold storage for 4hr, but were machine perfused (1h cold, oxygenated machine perfusion at low pressure, up to 3 mmHg) prior to graft implantation. The rat liver transplantation was performed according to Kamada et al. **1) HOPE** treated DCD liver grafts showed a significantly **reduced reperfusion injury** 12h after transplantation, as detected by improved liver function and integrity of different liver cell types (hepatocytes, Kupffer cells, SEC). **2)** Rats receiving non-perfused DCD livers demonstrated less body weight gain, increased bilirubin and severe intrahepatic biliary fibrosis 4 weeks after OLT. In contrast, HOPE treated DCD livers were **protected from biliary injury**, as detected by cholestasis parameter and histology (rodent MRI, CK-19, and Sirius red staining, Figure 1). We demonstrated that 1 hour cold oxygenated perfusion, after warm **and** cold ischemia, was highly effective against biliary injury.



Histology (sirius red) 4 weeks after liver transplantation

2.2.3 Reversible Portal Vein Embolization induces Liver Regeneration and Hypertrophy

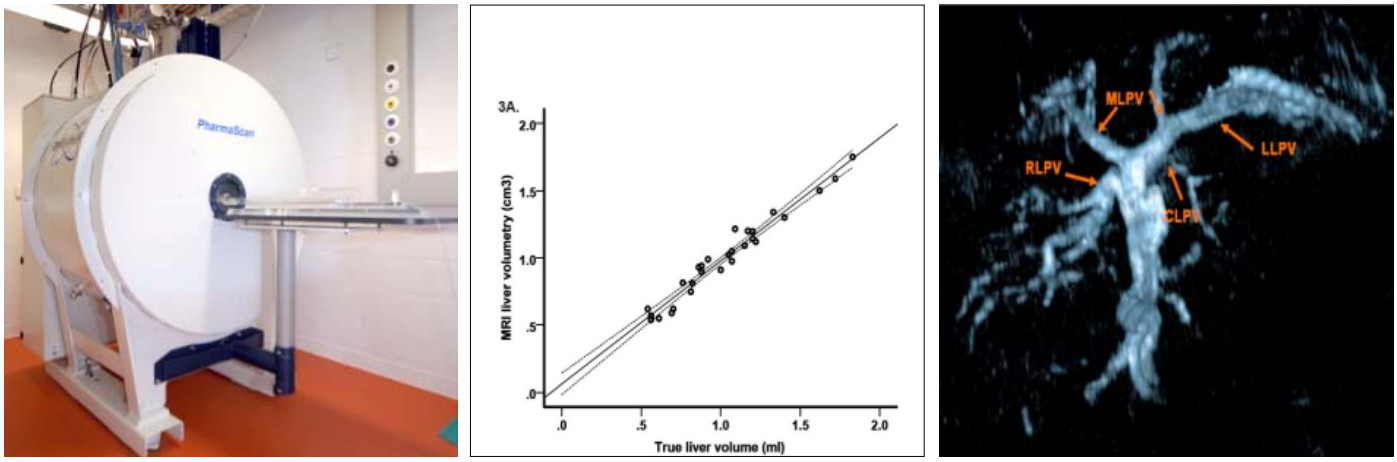
M. Lesurtel, E. Melloul, A. Vuck, N. Bain

Small animal magnetic resonance imaging (MRI): An efficient tool to assess liver volume and intrahepatic vascular anatomy

We demonstrated that the small animal MRI is an accurate tool to assess both liver hypertrophy during liver regeneration and corresponding modifications of intrahepatic vascular anatomy in mice. This method is non-invasive, reproducible and allows serial evaluation of the liver without the need to sacrifice animals. It is of great interest for the development of new agents to improve liver regeneration. In addition, it may help to better characterize morphological and vascular changes in other organs in experimental small animal models.

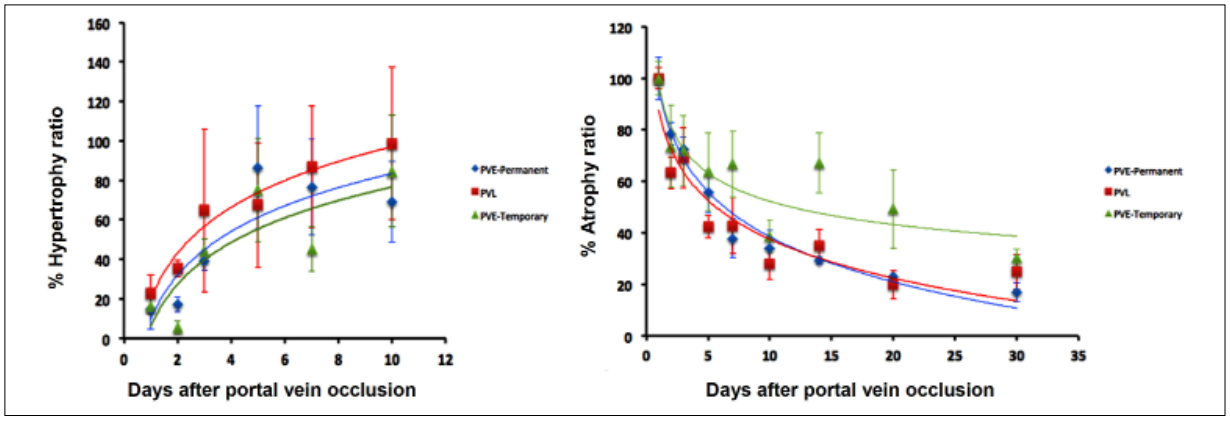
Permanent versus Temporary portal vein occlusion to induce liver hypertrophy: A comparative study.

The aims of this study were (1) to assess the efficacy of temporary portal vein embolization (PVE) compared to permanent occlusion of the portal vein with embospheres or portal vein ligature (PVL) on hypertrophy of the non-embolized liver lobes; (2) to observe the impact of temporary and permanent PVE on embolized liver lobes. 2 Although proximal and complete recanalization of the portal vein tributaries occurred 10 and 14 days after temporary PVE, the hypertrophy of the non-embolized lobes was similar than the hypertrophy induced by permanent PVE. In contrast, temporary PVE induced less atrophy of the embolized lobes than permanent PVE or PVL. The next step will be to assess the functional and morphological recovery of the embolized liver lobes after recanalization.



Left: Small animal MRI installed in our facilities (Pharmascan, Brucker Biospin®). Middle: Correlation between MRI liver volumetry vs. true liver volume measured by conventional methods in mice before and after hepatectomy. Right: MR angiography of the portal vein branches with three-dimension reconstruction using OSIRIX software

NB: Manuscript submitted to Radiology in March 2013.

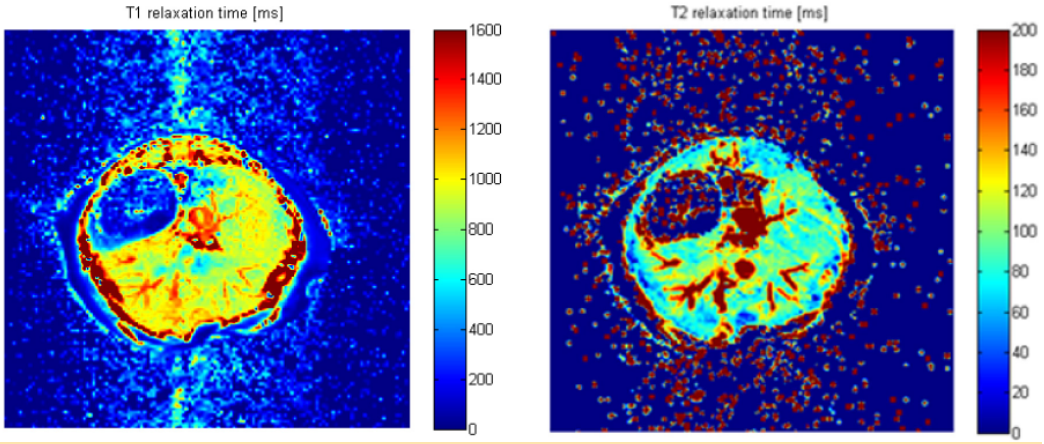


Left: Hypertrophy ratio of the non-embolized liver lobes after permanent PVE (bleu), PVL (red) and temporary PVE (green).

Right: Atrophy ratio of the embolized liver lobes after permanent PVE (blue), PVL (red) and temporary PVE (green).

Molecular Imaging of liver metabolic pathways after partial hepatectomy: Indicators of liver failure?

Post-hepatectomy liver failure (PLF) is associated with a failure of liver regeneration but the molecular mechanisms of PLF have not been elucidated yet. Using multi-modal molecular imaging techniques, the liver metabolism of glucose, pyruvate and ATP will be characterized during liver regeneration in a well-established mouse model of partial hepatectomy. We will test which biomarkers of molecular imaging are best suited to provide a quantitative measure for developing PLF. Probing of metabolic pathways in regenerating liver will be performed using hybrid positron-emission-tomography (PET)/magnetic resonance imaging techniques. Liver ATP synthesis will be measured with ^{31}P-magnetic resonance spectroscopy, pyruvatelactate metabolism with hyperpolarized [$^{1\text{-}13}$C]pyruvate spectroscopy, and glucose metabolism with dynamic ^{18}F-fluorodeoxyglucose (FDG) PET.



T1/T2 relaxation parameters of liver MRI representing characteristic tissue properties after partial hepatectomy in mice using a small animal MRI.

Collaborations:

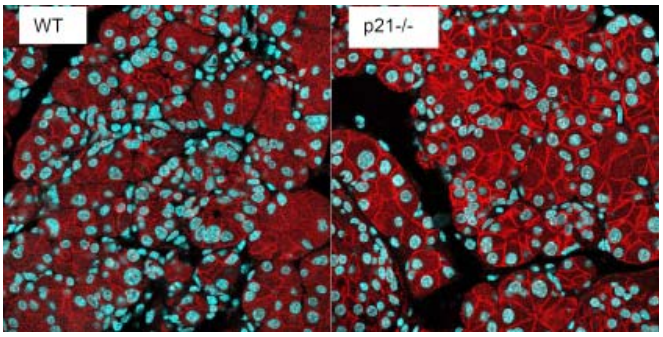
- Prof. Dr. Thomas Pfammatter, Department of Radiology, UniversityHospital Zurich
- PD Dr. Andreas Boss, Department of Radiology, UniversityHospital Zurich

2.2.4 Pancreatitis Research Laboratory

$p21^{WAF1/Cip1}$ down-regulation is critical for acinar-to-ductal metaplasia formation during pancreatitis

K. Grabliauskaite, E. Saponara, T. Reding Graf, M. Bain, S. Sonda and R. Graf

De-differentiation of pancreatic acinar cells into ductal-like lesions, a process defined as acinar-to-ductal metaplasia (ADM), is observed during organ regeneration following pancreatitis. In addition, ADM is found in association with pre-malignant PanIN lesions and correlates with an increased risk of pancreatic cancer. We found that $p21^{WAF1/Cip1}$ is strongly up-regulated during the regenerative phase and act as a gate-keeper of acinar cell dedifferentiation and formation of metaplastic epithelium by modulating β-catenin signalling.

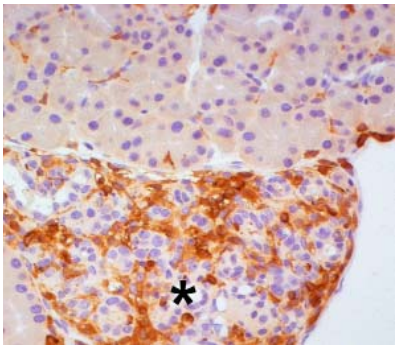


Expression of β-catenin in pancreatic acinar cells increases in absence of p21.

Inflammation contributes to the regression of acinar-to-ductal metaplasia during pancreatitis

K. Grabliauskaite, E. Saponara, T. Reding Graf, M. Bain, S. Sonda and R. Graf

A tight regulation of ADM regression is critical for the completion of regeneration of the injured pancreas and organ homeostasis. Our work revealed that ADM following pancreatitis or 60% pancreatectomy is associated with a strong inflammatory response, which is independent from the total level of inflammation in the pancreatic tissue. When mice with established ADM were treated with anti-inflammatory drugs, dissolution of ADM was prevented, suggesting that the immunity plays an active role in the regression of ADM.

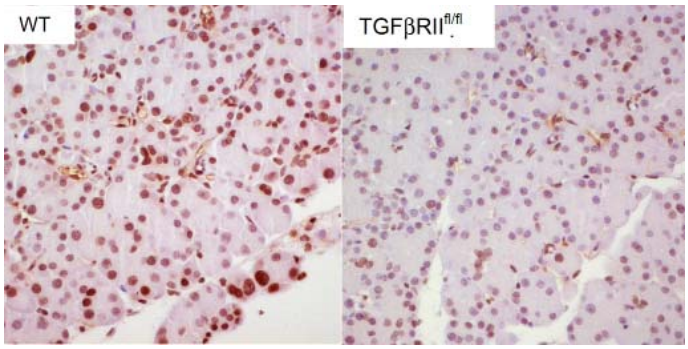


Acinar-to-ductal metaplasia (*) is more inflamed than the surrounding intact acinar tissue

Acinar specific TGF-β signaling regulates acinar cell regeneration

K. Grabliauskaite, T. Reding Graf, S. Sonda and R. Graf

TGFβ signalling is implicated in many pathophysiological functions of pancreatic cells. However, the function of TGFβ signalling is strongly context-dependent and an acinar cell specific role of this molecule in modulating regeneration has not been completely investigated before. By using mice deficient in TGFβ receptor II (TGFβ $RII^{fl/fl}$) exclusively in acinar cells, we showed that TGFβ signalling up-regulated p16INK4a expression, inhibited acinar cell cycle activation and prevented excessive ADM formation. Additionally, loss of TGFβ signalling in acinar cells potentiates fibrogenic processes during pancreatitis, suggesting the existence of a regulatory feedback between acinar and stellate cells.

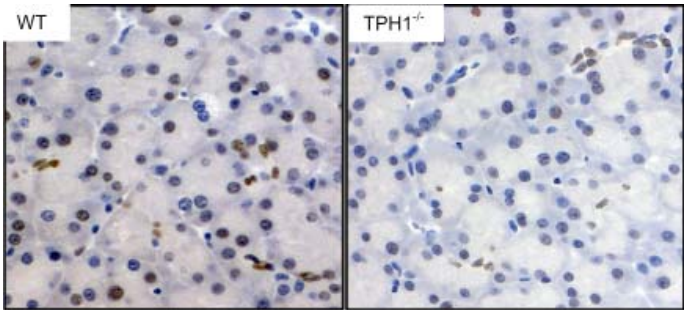


TGFβ signaling is required for up-regulation of p16INK4a in pancreatic acini following pancreatitis.

Serotonin regulates progenitor cell-based regeneration in the adult pancreas

E. Saponara, Y. Tian, K. Grabliauskaite, T. Reding Graf, M. Bain, S. Sonda and R. Graf

Regeneration of the pancreas appears to occur through two different programs, with the specific response being determined by the type and severity of the injury. Moderate kinds of pancreatic injuries, including 60% pancreatectomy, trigger a proliferation-based repair response, in which differentiated acinar cells divide to supply the additional cells necessary for tissue repair. In contrast, more significant injuries such as cerulein-induced pancreatitis, trigger a more powerful progenitor-cell based regenerative response. By using $TPH1^{-/-}$ mice with reduced peripheral levels of serotonin, we showed that this molecule is required for progenitor cell-based regeneration.

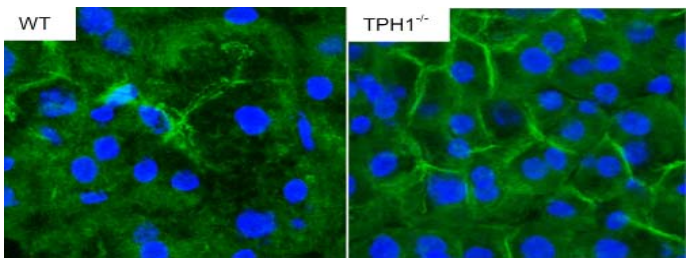


Lack of 5-HT in $TPH1^{-/-}$ mice prevents the up-regulation of the progenitor cell marker Sox9 in acinar cells following pancreatitis.

Serotonin mediates pancreatic acinar cell cytoskeletal remodeling in mice

E. Saponara, S. Sonda, K. Grabliauskaite, T. Reding Graf, R. Graf

In our previous study, we showed that serotonin is necessary for pancreatic acinar cell secretion in both physiological and pathological conditions. Since the secretory process is intrinsically dependent on cytoskeletal rearrangements, we now aim to study the effects of 5- HT on the regulation of cytoskeleton remodeling. Our data showed that serotonin modulates the dynamics of actin cytoskeleton and the localization of small GTPase proteins involved in cytoskeletal reorganization under both physiological and pathological conditions. Moreover, *in vivo* and *in vitro* experiments revealed serotonin regulates E-cadherin expression and cell to-cell adhesion.

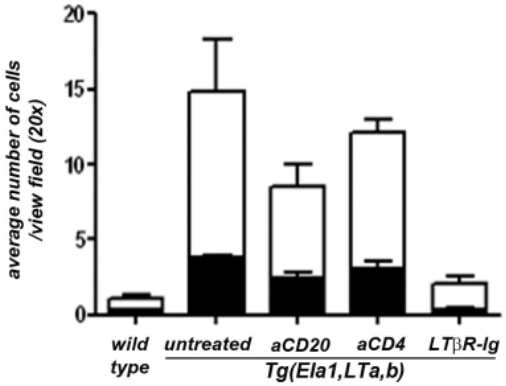


Expression of the cell adhesion protein E-cadherin in acinar cells increases in mice deficient for 5-HT ($TPH1^{-/-}$).

Comparative effectiveness of immune-cell depletion in the treatment of autoimmune pancreatitis

G. Seleznik, T. Reding Graf, S. Sonda, M. Heikenwälder, R. Graf

The long-term management of autoimmune pancreatitis (AIP) is still elusive. We previously demonstrated that acinar specific Lymphotoxin expression in mice Tg(Ela1-Ltα,β) induces autoimmunity with features reminiscent of human AIP. In our unique genetic mouse model of AIP, we now demonstrate that inhibition of Lymphotoxin beta receptor (LTβR) signalling pathway with LTβR-Ig is therapeutically superior to CD4+ T-cell and B cell depletion in decreasing autoantibody production, acinar-to-ductal metaplasia formation and NF-kB signaling activation. Therefore, inhibition of the LTβR-signalling pathway could become an alternative or supplementary approach for AIP treatment.

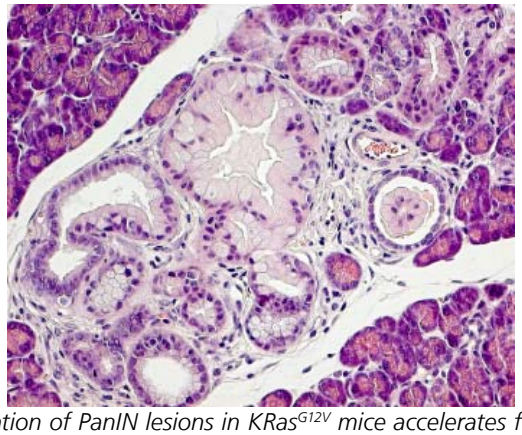


Treatment with LTβR-Ig efficiently prevents the activation of non-canonical NF-kb (RelB) signaliing.

Lymphotoxin beta receptor signalling pathway promotes initiation of pancreatic cancer

G. Seleznik, T. Reding Graf, S. Sonda, M. Heikenwälder, R. Graf

Pancreatic inflammation is a well-known risk factor for pancreatic ductal adenocarcinoma (PDAC) development in humans, but the underlining molecular mechanisms remain elusive. Here we established a new genetic model by intercrossing the commonly used p48$^{+/Cre}$; Kras$^{+/G12D}$ (KP) model for pancreatic tumorigenesis, to a novel transgenic mouse developing spontaneous pancreatic inflammation at an early age. Our data revealed that acinar cell expression of Lymphotoxin (LT$\alpha\beta$) dramatically accelerates the development of premalignant PanIN lesions and highlight the involvement of LTβ receptor signalling in the initiation of pancreatic cancer.



Formation of PanIN lesions in KRas^{G12V} mice accelerates following ectopic expression of lymphotoxin α,β in acinar cells

Collaborations/Sponsors:

- Prof. Dr. Mathias Heikenwälder, PhD, (TUM Munich)
- Prof. Dr. Adrian Hehl, MD, (University of Zurich)
- Prof. Dr. Achim Weber, MD, (University Hospital Zurich)

2.2.5 Bariatric Surgery

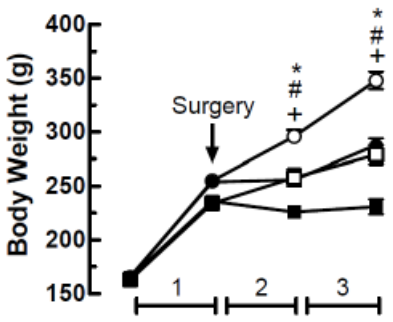
Estradiol increases body-weight loss and gut-peptide satiation after Roux-en-Y gastric bypass in ovariectomized rats

M. Bueter, K. Abegg, N. Theis, L. Asarian, M. Schiesser

More than 85% of patients undergoing bariatric surgery are female. We therefore investigated the effects of Roux-en-Y gastric bypass (RYGB) surgery on body weight and glucagon-like peptide-1 (GLP-1)- and cholecystokinin (CCK)-induced satiation in a rat model of menopause.

We were able to show in ovariectomized rats , that (1) RYGB-induced body-weight loss and (2) the satiating efficacy of endogenous glucagonlike peptide-1 (GLP-1) and cholecystokinin (CCK) satiation were significantly increased in estradiol-treated rats.

Our data suggest that RYGB may be more effective in healthy premenopausal women than in postmenopausal women who do not receive hormone-replacement therapy or in women with reduced estrogen levels, such as patients with polycystic ovary syndrome.



*Body weights (A) and food intakes (B) following RYGB or sham operation in ovariectomized rats that were treated with estradiol (E2) or the oil vehicle (OIL). #SHAM-OIL vs. RYGB-OIL; *RYGB-OIL vs. RYGB-E2; +SHAM-OIL vs. SHAM-E2; Ps<0.01 for body-weight gains and Ps<0.05 for food intakes.*

Acute peripheral GLP-1 Receptor Agonism or Antagonism does not alter Energy Expenditure in Rats after Roux-en-Y Gastric Bypass

M. Bueter, K. Abegg, N. Theis, L. Asarian, M. Schiesser

We investigated if increased glucagon-like peptide-1 (GLP-1) levels are involved in the alterations in energy expenditure after Roux-en-Y gastric bypass surgery (RYGB).We found that acute subcutaneous administration of the GLP-1 antagonist Exendin (9-39) increased food intake in RYGB, but that there was no effect on energy expenditure.

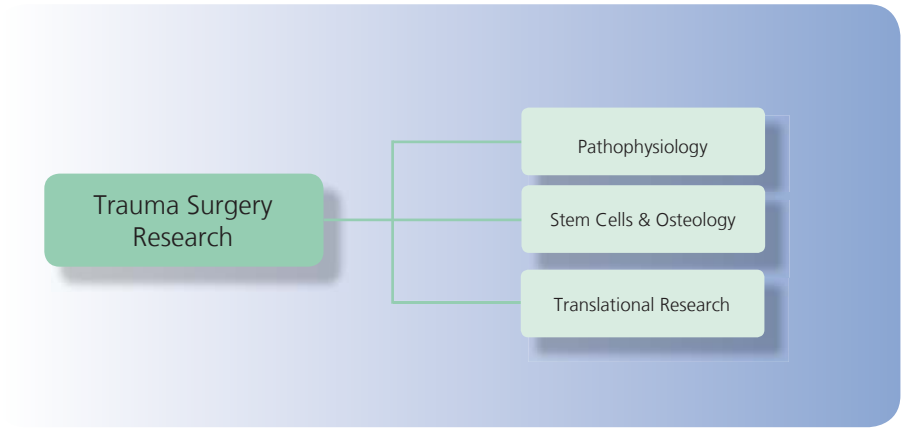
Similarly, GLP-1 agonist Exendin-4 (Ex-4, 5 µg/kg) decreased food intake in RYGB, but it did not modulate energy expenditure in any surgical group.

We conclude that acute modulation of GLP-1 signaling is not directly involved in altered energy expenditure after RYGB surgery in rats.

Collaborations/Sponsors:

- Prof. Dr. vet. Thomas Lutz (Institute of Veterinary Physiology, University of Zurich)
- Prof. Dr. Alan Spector, MD, (Florida State University, USA)

2.3 Trauma Surgery Research



Prof. Dr.
Guido A. Wanner,
MD

Prof. Dr.
Hans-Peter
Simmen,
MD, Director

PD Dr.
Paolo Cinelli,
PhD

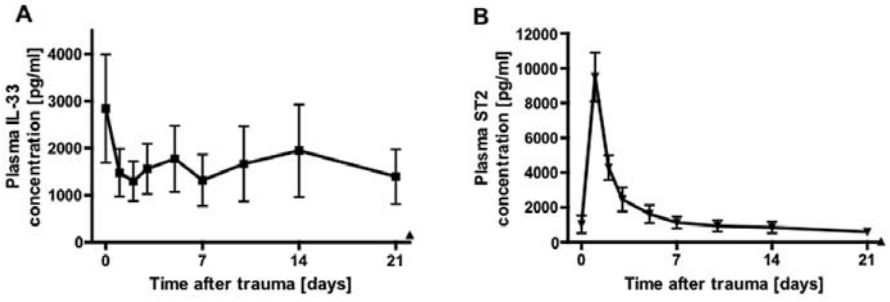
Prof. Dr.
Clément Werner,
MD

2.3.1 Pathophysiology

Interleukin-33 and its soluble receptor ST2 – novel players in trauma-induced systemic inflammations

D. Rittirsch, V. Schoenborn, S. Märsmann, G.A. Wanner

The inflammatory response following trauma is highly complex and still inadequately understood. Endogenous danger signals (alarmins) play a crucial role in the initiation of the immune response. The novel cytokine interleukin-33 (IL-33) is known to act as an alarmin in various inflammatory conditions. Its soluble decoy receptor sST2 functions as an endogenous antagonist of IL-33. Aim of the study was to investigate the role of IL-33 and sST2 in systemic inflammation in patients with multiple injuries. We could demonstrate for the first time that IL-33 and its soluble receptor sST2 are released during the early phase after multisystem injury. While IL-33 peaks within hours after trauma, the kinetics of sST2 release differ, with peak levels on d1. Plasma concentrations of sST2 during the early phase reflect the severity of injury and are associated with the development of sepsis. Intriguingly, the data suggest that the spleen may represent a source for sST2 and/or may be involved in the regulation of sST2 release during the late phase after trauma.



Time course (day 0 - 21) of interleukin-33 (IL-33) (A) and soluble ST2 (sST2) (B) in plasma from patients with multiple injury (injury severity score ISS ≥ 17 points, n=32). ▲= healthy volunteers (n=10).

In conclusion, these findings suggest that IL-33 and sST2 contribute to systemic inflammation after trauma.

PD Dr.
Dieter Cadosch,
MD

Dr.
Daniel Rittirsch,
MD

Dr.
Carola Würgler-
Hauri,
MD

Dr.
Veit Schönborn,
MD

Dr.
Kai Sprengel,
MD

Dr.
Sebastian Günkel,
MD

Dr.
Max Joseph
Scheyerer,
MD

Dr.
Michael Zürcher,
MD

Dr.
Marius König,
MD

Dr.
Stefanie Hirsiger,
MD

Dr.
Thorsten
Jentzsch,
MD

Dr.
Stefan
Zimmermann,
MD


Dr.
Helmut
Wegmann,
MD


Dr.
Florian Grubhofer,
MD


Dr.
Pavel Zwolak,
MD


Dr.
Shuping Gao,
PhD


Sonja Hemmi,
Lab. Technician


Schirin Ibrahim

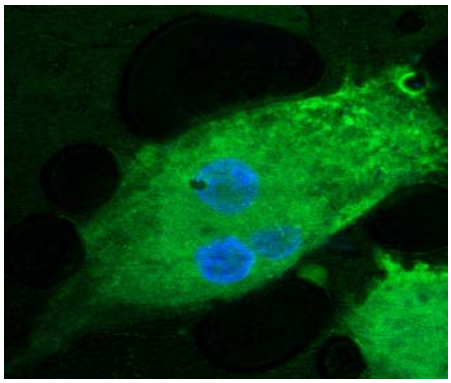

Sonja Märsmann,
Lab. Technician

2.3.2 Stem Cells & Osteology

Adverse reactions to metal ions

D. Cadosch and H.-P. Simmen

Although many studies have investigated the role of metallic and non-metallic wear particles in osteoclastogenesis and aseptic loosening, very little is known about the effects of metal ions released by biocorrosion from the implant surface. At the bone metal interface, metal ions may directly interact with bone cells accelerating osteoclastic bone resorption and/or inhibiting the function of osteoblasts. Our projects aim to investigate the effects of the released metal ions on bone metabolism and their involvement in the pathophysiological mechanisms of increased osteolysis. Currently we are investigating the effects of vanadium 4+ and 5+ ions on the differentiation and activation of human osteoclasts *in vitro*. The results suggest that vanadium has an inhibitory effect on osteoclastic differentiation and activation without affecting cellular viability.

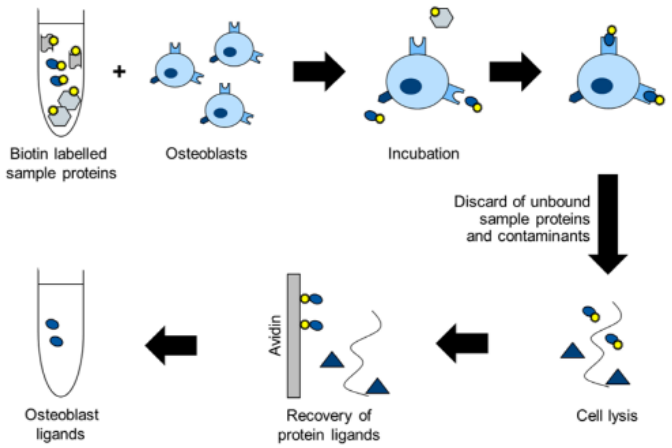


Multiphoton confocal fluorescence microscopy image (optical sections through the ''nuclear level'') showing human osteoclasts with intracellular metal ions complexes detected with Newport Green DCF diacetate ester (green color). Nuclei are stained in blue with DAPI.

Identification of humoral factors responsible for an enhanced osteogenicity in patients with a severe traumatic brain injury

D. Cadosch, H.-P. Simmen, P. Cinelli and G. Wanner

It is nowadays well established that severe traumatic brain injury (TBI) are associated with an accelerated fracture healing with hypertrophic callus formation and an increased incidence of heterotopic ossification. It is suggested that osteoinductive factor(s) are released by the injured brain into the blood circulation and act peripherally on the affected soft tissue. We were previously able to demonstrate the *in vitro* osteogenic potential of cerebrospinal fluid and serum derived from patients with a severe TBI. Hence, since then our research aimed to identify the assumed osteogenic factors. Using a novel proteomic approach, we found clear-cut differences in the pattern of proteins in two-dimensional gels between TBI and control patients without TBI. The identification of these factors would enable a better understanding of the pathophysiological mechanisms of fracture healing. Foremost, it would offer the possibility of developing novel therapies, which would improve and enhance fracture healing.

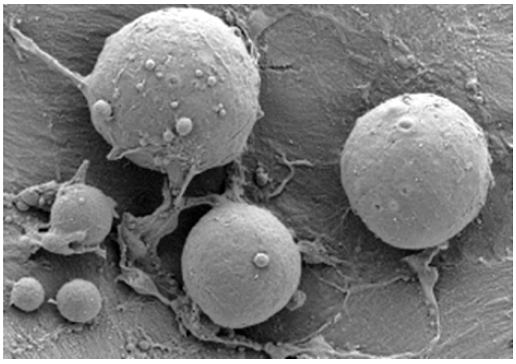


The figure shows the method of cell-based protein enrichment developed by our group. The method allows the isolation of all proteins that have a functional affinity for the surface receptors of a target cell line (e.g. human osteoblasts). Biotin is represented as yellow dot; structural osteoblastic proteins are represented as blue triangles.

Perivascular stem cells for bone tissue engineering

D. Cadosch, F. Grubhofer, G.-A. Wanner and P. Cinelli

Tissue engineering research has endeavored to search for novel sources of stem cells other than bone marrow mesenchymal stem cells (MSCs) for bone regeneration and repair. Pericytes has recently been identified as a primitive origin of human MSCs. We established an effective protocol to purify CD146+ NG2+ CD34- CD45-, pericytes from adipose tissue using a magnetic-activated cell sorting (MACS) method. The osteogenic potential of the pericytes will now be investigated *in vivo* using a robust "critical bone defect" model with and without scaffold and Nell-1, a novel osteoinductive growth factors.



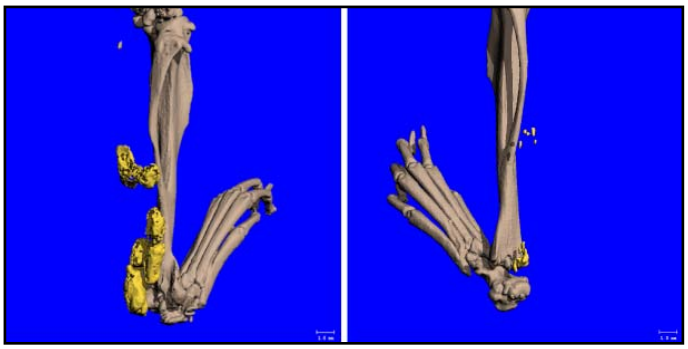
The figure shows CD146+ NG2+ CD34-, CD45-, pericytes growing in vitro *on a human cancellous bone chip (hCBC) scaffold.*

Heterotopic Ossification: New Approaches continued

S.J. Zimmermann, C.C. Würgler-Hauri, M. Scheyerer, H.-P. Simmen, C.M.L. Werner

Heterotopic ossification (HO) is a problem widely encountered by orthopedic and trauma surgeons. HO may result in joint contracture, pain or even spasticity and neurovascular compression leading to significant disability. Patients with high-grade ossification frequently necessitate reoperation thereby largely increasing the costs of treatment. This is why effective means of prevention are of great importance in a clinical setting.

The exact mechanism leading to HO formation is not completely understood. One possible explanation is that hypoxic stress induces angiogenic stimulators, which trigger stem cells to differentiate to chondroytes with subsequent heterotopic bone formation.



Typical location and component arrangement in control (left) and Imatinib group (right)

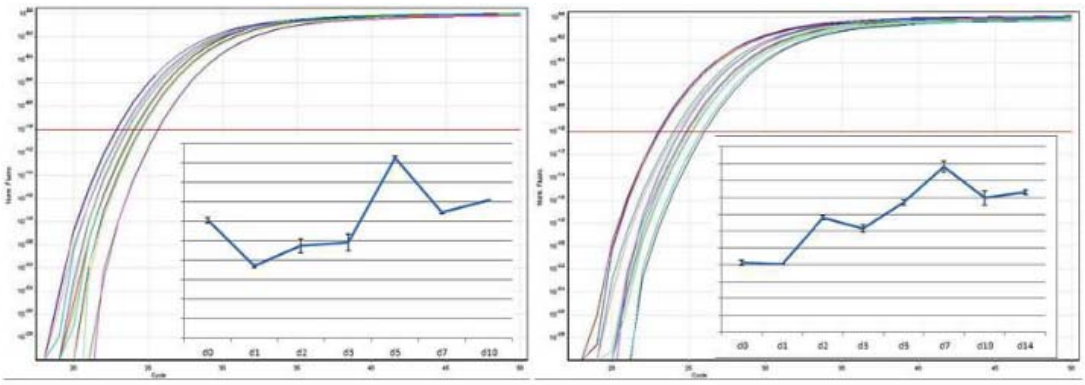
We investigated the effect of inhibited neovascularization of hypoxic tissue on the development of heterotopic ossification. A standardized animal model to produce heterotopic ossification by means of an Achilles tenotomy was chosen. All mice underwent bilateral Achilles tendon tenotomy and were divided into two groups: The control group underwent Achilles tenotomy only. The Imatinib group received Imatinib Mesylate orally by gavage once a day for a duration of six weeks. After 10 weeks the limbs were harvested and Micro CT was performed. Heterotopic bone volume was then identified in 3d images and statistical analysis was performed. A significant reduction of 85% could be found in the Imatinib group. Despite this encouraging results the potential effect of Imatinib on impairing the fracture healing has still to be determined. Future studies involving standardized fracture models will aim at investigating this matter.

2.3.3 Translational Research

Transcriptomic profiling in severely injured patients, opening "the window of opportunity"

V. Schoenborn, D. Rittirsch, S. Günkel, P. Cinelli, M. Bauer, G.A. Wanner

Most patients with multiple trauma develop post-traumatic complications such as a systemic inflammatory response syndrome (SIRS), sepsis and severe multiple organ dysfunction or - failure (MODS/MOF). Treatment of patients with severe trauma is performed by two alternative approaches: In "Early total care" all necessary operations are carried out immediately, whereas in the "Damage control" concept the patient is stabilized first and final operations, which might present a second stress/trauma for the patient, are performed some days (4-7 days) later during the "window of opportunity". However up to date no exact measures exist to precisely define this time point, which should be in an optimal way during a balanced phase of inflammatory and anti-inflammatory reaction. We performed a wholegenome gene expression analysis to get an insight into the complex mechanisms of the proand anti-inflammatory reaction followed by trauma. More than 100 severely injured patients with an injury severity score (ISS) >16 are enrolled in the study until today.
We identified a number of promising, not yet describe genes which exhibit changes in expression and might play an important role in the inflammatory response. We validated the data by quantitative RT-PCR and we are currently designing a QRT-PCR based clinical diagnostic test for sepsis/SIRS for the evaluation of our patients.



Representative expression changes of TNFα in two patients at different time points upon injury.

Collaborations/Sponsors:

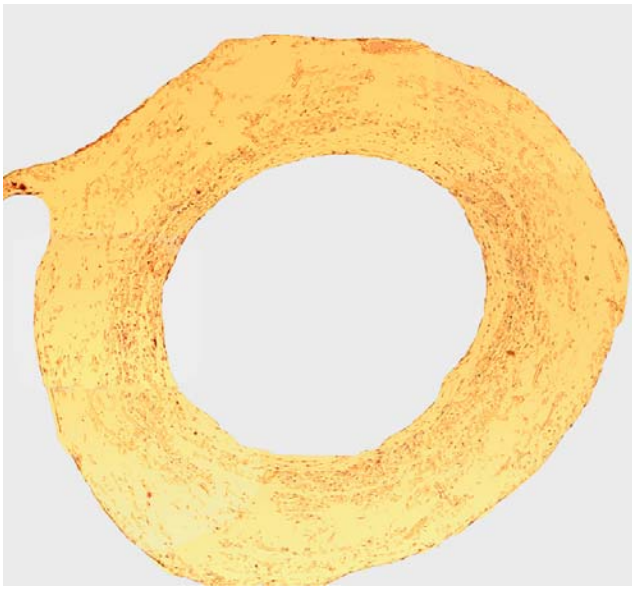
- Clinical Trials Center, UniversityHospital Zurich
- Brigitte von Rechenberg, Musculoskeletal Research Unit, Vetsuisse Faculty, University of Zurich
- Lukas Sommer, Institute of Anatomy, University of Zurich
- Center for Applied Biotechnology and Molecular Medicine (CABMM), University of Zurich
- Armin Curt, Spinal Cord Injury Center, University of Zurich and University Hospital Balgrist
- Orthopedic Research Laboratory, Biomechanics, University Hospital Balgrist, Zurich
- Beatrice Beck-Schimmer, Institute of Anesthesiology, UniversityHospital Zurich
- Institute for Biomechanics, ETH, Zurich
- Michael Bauer, Institute for Anesthesiology and Intensive Care Medicine, University Hospital Jena, Germany
- Markus Huber-Lang, Dept. of Traumatology, Hand-, Plastic and Reconstructive Surgery, University Hospital Ulm, Germany
- Jan Schwab, Klinik und Poliklinik für Neurologie & Experimentelle Neurologie, Charité Universitätsmedizin Berlin
- Michael Flierl, Philip Stahel, Dept. of Orthopaedic Surgery, Denver Health Medical Center, USA
- Peter A. Ward, Dept. of Pathology, University of Michigan Medical School, Ann Arbor, USA
- Alessio Fasano, Mucosal Biology Research Center, University of Maryland, Baltimore, USA
- AO Clinical Investigation and Documentation (AOCID)

2.4 Cooperation Trauma Surgery and Plastic, Hand & Reconstructive Surgery Research

Tissue Engineering of Bone Grafts using ASC-derived Osteoblasts and ASC-derived Endothelial Cells Co-cultured on a Biomimetic Nanocomposite

P. Cinelli , M. Calcagni, M. Welti, S. Hemmi, N. Hild, W. Stark, G. Meier-Bürgisser, S. Gao, G. Wanner, J. Buschmann

Tissue-engineered bone produced by using mesenchymal stem cells (MSCs) is a promising method for the rehabilitation of acquired bone defects. However, generating a vascular supply to the engineered graft remains a major challenge. In order to optimize the preparation of vascularized tissue engineered bone we expanded MSCs isolated from adipose tissue to form osteoblasts *in vitro*, in parallel a second portion of MSCs was directed to differentiate into endothelial cells. Both cell types were then seeded onto a biodegradable poly (l-lactide-coglycolide)- tricalcium phosphate (PLGA-aCaP)-based scaffold and cultivated further for two weeks (control: PLGA). Our preliminary results confirm that both endothelial cells and osteoblasts are able to successfully colonize and expand in the PLGA-aCaP scaffold indicating that this approach might be beneficial for future therapeutic applications.



Cross section of a PLGA-aCaP scaffold seeded with endothelial cells and osteoblasts (H&E staining, 25 x magnification)

PD Dr.
Paolo Cinelli,
PhD

Dr.
Johanna
Buschmann,
PhD

Prof. Dr.
Guido A. Wanner,
MD

PD Dr.
Maurizio
Calcagni,
MD

Dr.
Shuping Gao,
PhD

Gabriella Meier-
Bürgisser,
M.Sc.

Sonja Hemmi,
Lab. Technician

Manfred Welti,
Lab. Technician

2.5 Plastic, Hand & Reconstructive Surgery Research


PD Dr.
Maurizio Calcagni,
MD


Prof. Dr.
Pietro Giovanoli,
MD, Director


PD Dr.
Nicole
Lindenblatt,
MD


Dr.
Johanna
Buschmann,
PhD


PD Dr.
Claudio Contaldo,
MD


Dr. Dr. dent.
Marius Bredell,
MD


Dr.
Juliane Finke,
MD


Dr.
Tatjana Lanaras,
MD


Alicia Hegglin,
M.Sc.

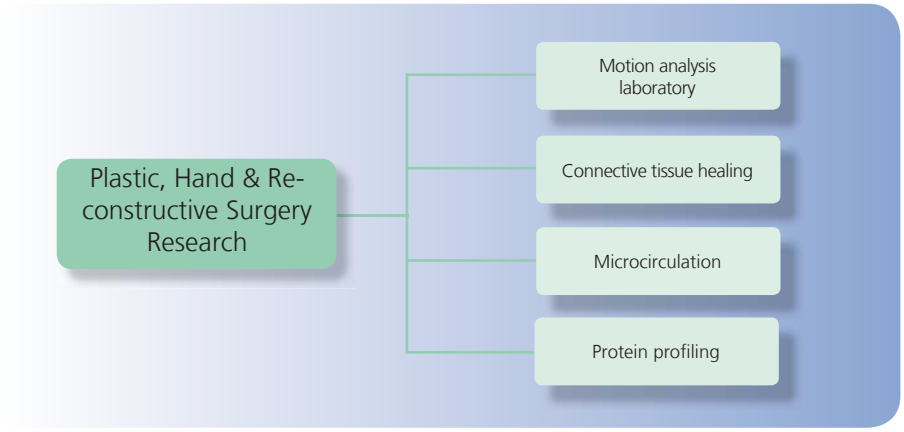

Angela Müller,
med. Student


Gabriella Meier-
Bürgisser,
M.Sc.


Flora Nicholls,
Dipl. nat.


Dr.
Dominik Högger,
MD


Conny Waschkies,
Lab. Technician



2.5.1 Tissue Engineering

In vivo high field MRI and histological analysis of artificial organs grown on the chorioallantoic membrane of the chick embryo: quantification of the perfusion capacity.

F. Kivrak-Pfiffner, C. Waschkies, Y. Tian, M. Calcagni, P. Giovanoli and J. Buschmann

In this study, we determined T_1 relaxation rates in polyurethane foam scaffolds grown for 1 week on the chorioallantoic membrane of the chick embryo (CAM) before and after i.v. injection of Dotarem into the capillary system of the chick embryo. On incubation day 7, egg shells of fertilized eggs were opened and the scaffolds carefully placed on top of the CAM as shown in *Figure 1*. After one week on incubation day 14, *in vivo* high field MRI scans were made focusing the scaffold; once not injected (delivering R_{10} values) and then after i.v. injection delivering R_1 values (*Figure 2*). R_1 values were significantly higher than R_{10} values (*Figure 3*). After formalin fixation overnight, scaffolds were extracted. Relaxation rates were correlated to the capillary density scored semi-quantitatively in 4 succeeding histological sections separated by 1 mm and stained by H&E and immunohistochemistry (von Willebrand Factor). This study has been funded by *Matching Fund 2011*.

Figure 1: DegraPol foam carefully placed on top of the CAM.

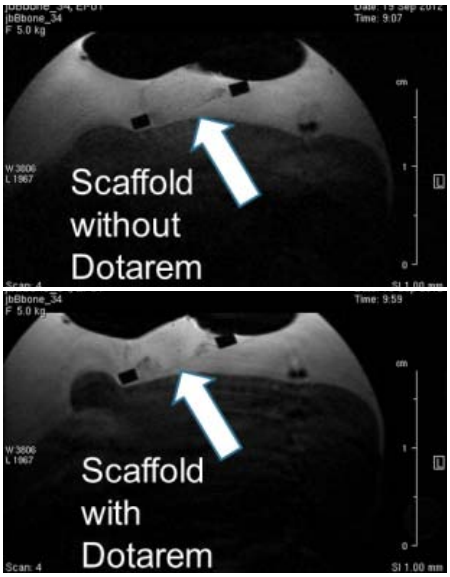


Figure 2: Cross section of a PLGA-TCP scaffold seeded with endothelial cells and osteoblasts (H&E staining, 25 x magnification)

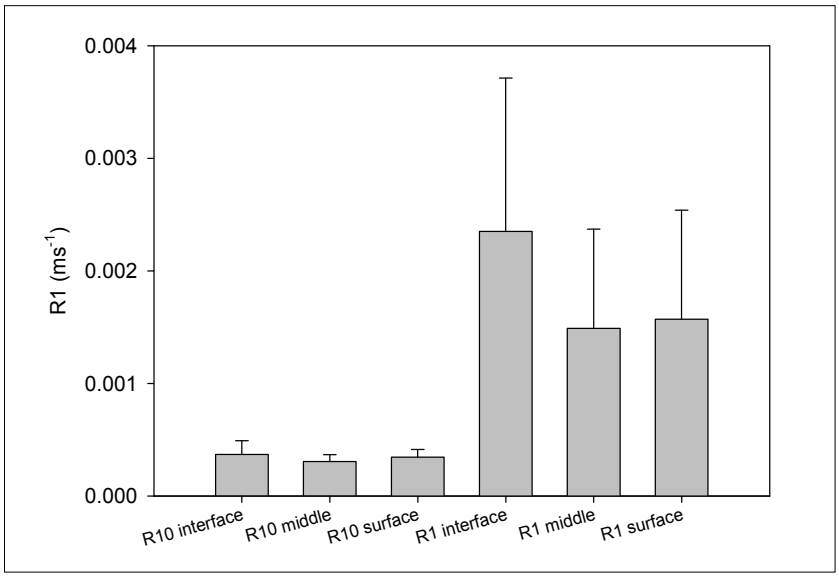


Figure 3: Relaxation rates (R1) found in the scaffolds at the interface to the CAM, in the middle and at the surface; compared to background relaxation rates (R10).

Visualization of PDGF-BB on electrospun DegraPol scaffolds by immunostaining.

O. Evrova, S. Nötzli, V. Milleret, M. Calcagni, E. Bonavoglia, P. Giovanoli, J. Buschmann

Electrospun scaffolds from different polymers can act as carriers of growth factors and thus allow for different applications in the area of tissue engineering and regenerative medicine. For optimal use of these scaffolds and proper healing processes, appropriate release kinetics of the growth factors present on the scaffolds is necessary. Loading the scaffolds with growth factors can be realized by different immobilization techniques resulting either in covalent crosslinking, physical ab/adsorption, binding with the help of heparin or others.

Electrospun meshes of DegraPol® (DP) scaffolds are used as carriers for localized, slow and adjusted delivery of platelet derived growth factor (PDGF-BB) to accelerate tendon rupture repair. One first step to verify the presence of this growth factor on electrospun DP fibers is to visualize PDGF-BB on the scaffold by immunostaining. Results obtained as microscopic images *(Figure 1 A-D)* show that PDGF-BB can be nicely visualized with this method of immunostaining. *Figure 1A* and *1B* show that there is no background binding of the primary and secondary antibody and thus producing background signal that would interfere with the real presence of PDGF-BB. PDGF-BB was present more on the scaffold when dripped on top *(Figure 1C)* then when added by means of emulsion and later electrospinning.

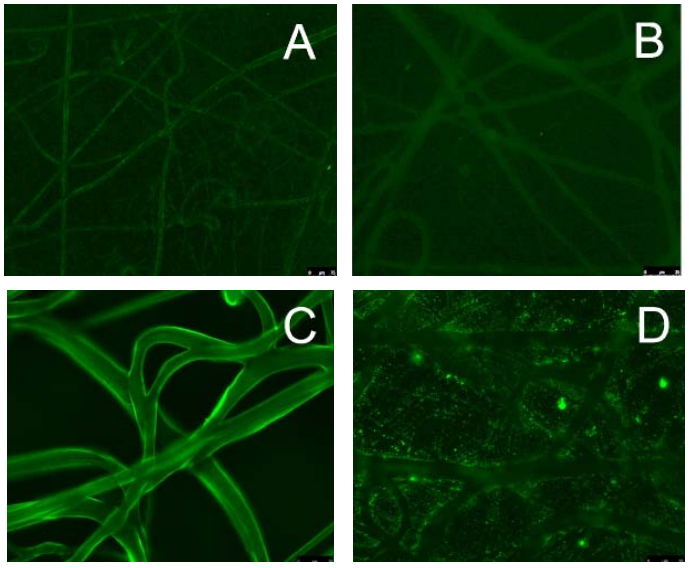


Figure 1. A: DegraPol® scaffold without any PDGF-BB (negative control). B: DegraPol® scaffold without any PDGF-BB (negative control - incubated only with secondary antibody, to eliminate any background signal if present). C: DegraPol® scaffold with PDGF-BB dripped on top of pure DegraPol® fibers. D: DegraPol® scaffold electrospun with PDGF-BB present from emulsion.

2.5.2 Connective Tissue Healing

Correlation of high-frequency ultrasound images with histomorphometric analysis of healing rabbit achilles tendon ruptures three and six weeks post-surgery

J. Buschmann, G. Puippe, G. Meier Bürgisser, E. Bonavoglia, P. Neuenschwander, P. Giovanoli and M. Calcagni

The purpose of this study was to correlate static and dynamic High-Frequency Ultrasound of healing rabbit Achilles tendons with an in-depth histomorphometric analysis of the healing tissue at three and six weeks post-surgery. Twelve New Zealand White rabbits received a clean-cut Achilles tendon laceration and were repaired with a 4-strand suture. Six rabbits got additionally a tight polymer tube at the repair site. Tendons were analysed by static and dynamic Ultrasound and Power Doppler Ultrasound (control: healthy contralateral legs). The ultrasound outcome was correlated to the tendon shape, tenocyte and tenoblast density, tenocyte and tenoblast nuclei width, collagen fibre orientation and adhesion extent. All repaired tendons showed a spindle-like morphology in the ultrasound, corresponding to the same shape caused by the swollen epitenon (histology). Prediction of adhesion formation by dynamic Ultrasound assessment was confirmed by determining the contact region of the newly formed tissue to the surrounding tissue (histology). Hyperechogenic areas in ultrasound images indicating an interrupted reflex corresponded to acellular zones with aligned fibres and hypoechogenic zones to not yet oriented fibres and cell rich areas (Figure 1). These findings support the usefulness of High Field Ultrasound as a non-invasive analytical tool for monitoring the healing of tendons. The study is funded by the *Fonds für Medizinische Forschung*, the *Wolfermann-Nägeli foundation* and *ab medica,* Italy.

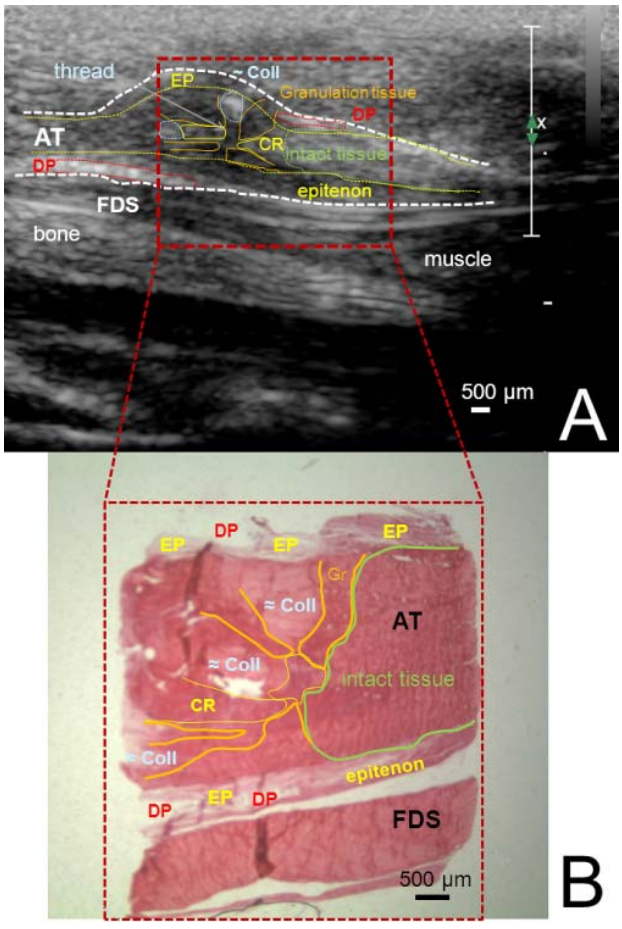


Figure 1: Ultrasound image (A) and corresponding Picosirius Red stained histological sections at 8 x magnification (B) of rabbit Achilles tendon ruptures repaired with a 4-strand Becker suture and a DP tube evaluated 3 weeks post-operation. Key: AT = Achilles tendon (white dotted lines), FDS = flexor digitorum superficialis, DP = DegraPol tube (red dotted lines), EP = epitenon (yellow dotted lines), CR = cell rich tissue (yellow solid lines), Gr = granulation tissue (orange dotted lines), ~ Coll = acellular zone (light blue dotted lines).

Adhesion formation of repaired rabbit Achilles tendon with and without DegraPol tube

G. Meier-Bürgisser, M. Calcagni, E. Bonavoglia, P. Giovanoli, J. Buschmann

In (flexor) tendon rupture repair, there are still open problems up-to-date: adhesion and rup-ture in the early healing phase with a reoperation rate of 7 – 15 % leading to increased work disability and costs. On the one hand side, the repaired tendons should have high primary re-pair strength for early active post-operative motion, and on the other hand side, the repair site should be flat in order to allow the tendon to glide smoothly in the tendon sheath. According to Kuwata et al., optimum primary repair strength requires multi-strand locking loops and cross-stitch epitendinous sutures. However, such repair techniques lead to bulging at the repair site and thus to adhesion during the healing process.

Considering these problems, a polymer device which bags the repaired tendon tightly and has a flat outer surface would probably help to reduce the adhesion caused by a rough and large primary cross-sectional area at the repair site. In addition, such a flattening tube may act as a potential carrier device and be supplemented with bioactive substances or stem cells in order to stimulate the healing process *in situ*.

As a consequence, we developed a potential carrier system which is able to do both; flatten the repair site and deliver growth factors, cytokines or other stimuli to the repair site. DegraPol® tubes were implanted around transected and conventionally sutured rabbit Achilles tendons and adhesion formation was quantified 6 weeks post-operatively according to Tan et al. *(Tan et al., 2010)*. DegraPol tubes applied in addition to a 4-strand Becker suture were found to significantly reduce adhesion formation compared to conventionally sutured rabbit Achilles tendons (mere 4-strand Becker sutures).

The study is funded by the *Fonds für Medizinische Forschung*, the *Wolfermann-Nägeli foundation* and *ab medica*, Italy.

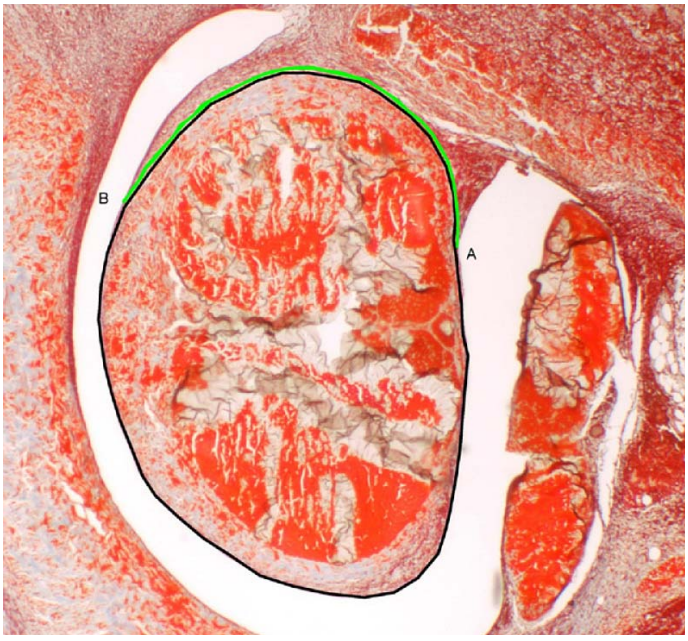


Figure 1: Analysis of contact region. Contact region between newly built fibrous tissue and tendon tissue (green line between A and B) and whole contour of the tendon (black line). Adhesion extent is calculated by dividing the length of the green line by the length of the black line
(method taken from Tan *et al*, Effects of Nonsteroidal Anti-Inflammatory Drugs on Flexor Tendon Adhesion*, JHS 35A, June 2010).*

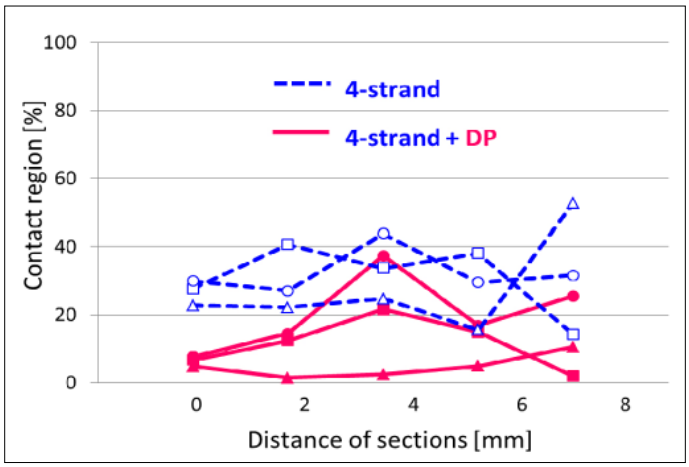


Figure 2: Adhesion extent at the repair site of conventionally sutured rabbit Achilles tendons (4-strand) and of 4-strand sutured tendons that received additionally a DegraPol tube (4-strand + DP).While 4-strand sutured tendons exhibited on average 30% contact to the surrounding tissue over a distance of 7 mm at the repair site, the tendons that were 4-strand sutured and got a DegraPol tube showed a significantly lower contact region of on average 12% ($p < 0.001$).

2.5.3 Microcirculation

In vivo evaluation of wound bed reaction and graft performance after cold skin graft storage – potential new targets for skin tissue engineering

A. Hegglin, K. Kornmann, K. Kerl, M. Calcagni, P. Giovanoli, N. Lindenblatt

In plastic surgery surplus harvested skin grafts are routinely stored at 4-6°C for several days. The purpose of this study was to evaluate the influence of storage on human split-thickness skin grafts (STSG) performance in an *in vivo* setting in order to identify potential mechanisms for use in skin tissue engineering. Human full-thickness skin grafts (FTSGs) and STSGs were transplanted into the modified dorsal skinfold chamber of mice and intravital microscopy was performed *(Fig. 1)*.

STSGs were harvested 10 days after transplantation for immunohistochemistry (human CD31, murine CD31, Ki67, Tunel). The IVM showed no differences in the host angiogenic response between fresh and preserved grafts. However, STSGs and FTSGs exhibited a trend towards different timing and extent in capillary widening and capillary bud formation. Preservation had no influence on graft score and quality before transplantation, but fresh grafts showed better quality 10 days after transplantation than 7 days preserved grafts *(Fig. 2)*. Proliferation and apoptosis as well as host capillary in-growth and graft capillary degeneration were equal in both groups. These results indicate that under extreme conditions, cutaneous cells may activate some protective mechanisms which allow them to reestablish normal function. However, rewarming may disclose underlying tissue damage not seen during cooling.

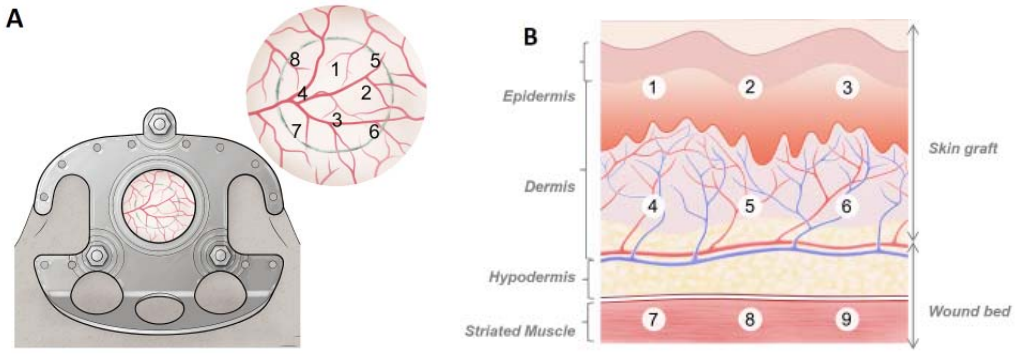


Fig. 1: (A) Intravital microscopy of the wound bed. Schematic view of the respective areas studied in the center and the periphery of the wound bed (1-8). (B) Areas of histological determination of proliferation and apoptosis rate in the upper dermis (1-3), lower dermis (4-6) of the graft and the host wound bed (7-9).

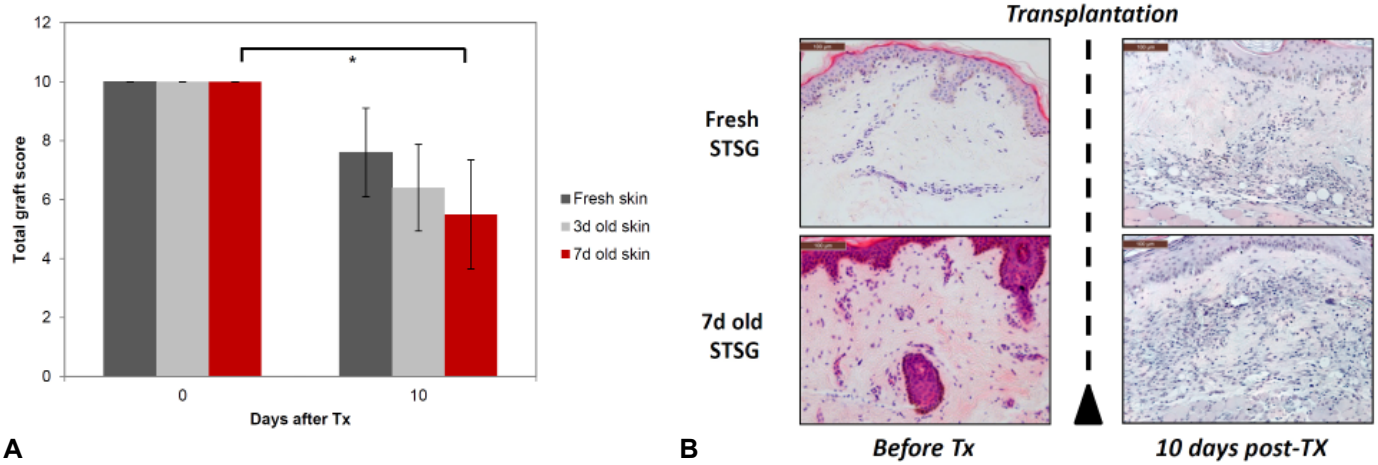


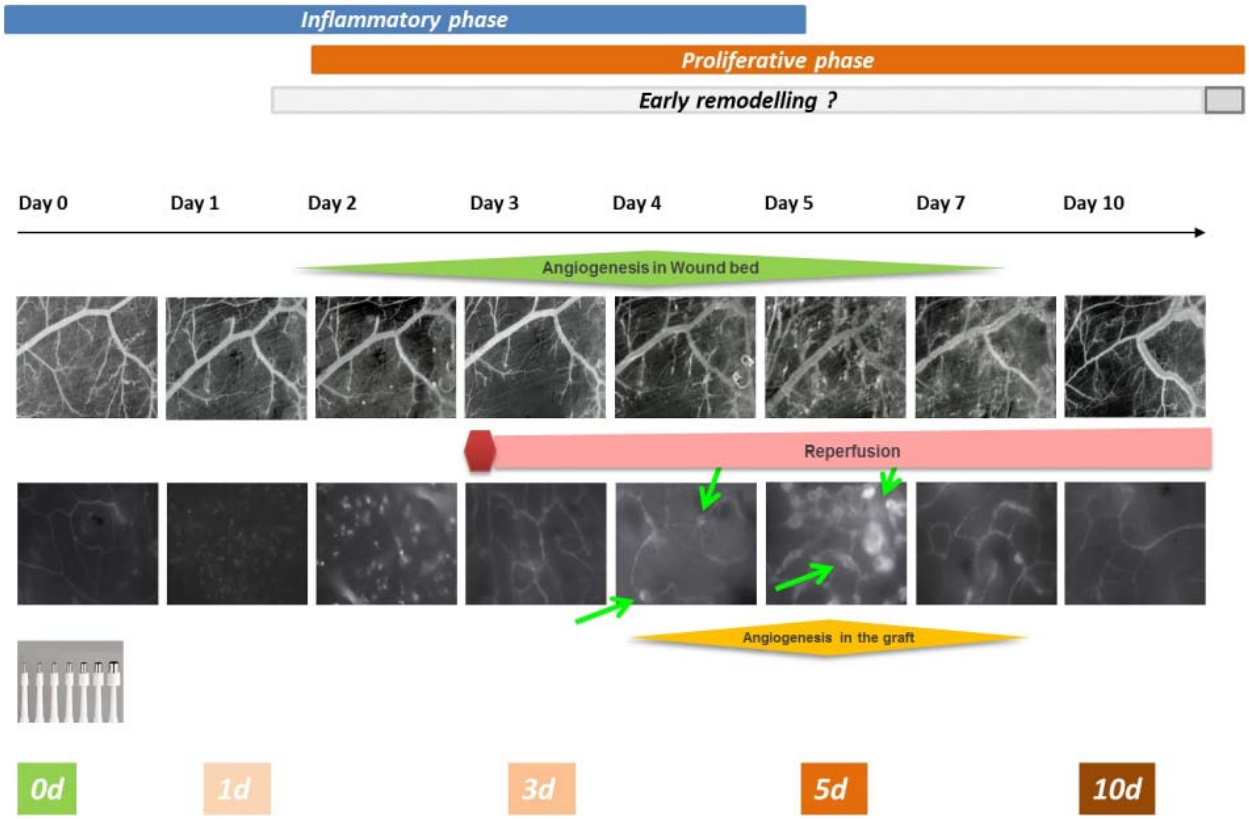
*Fig. 2: Histological evaluation of fresh and stored human STSGs 10 days after transplantation. (A) Blinded pathological skin quality evaluation. * p≤0.05 day 0 vs. day 10. (B) Representative HE sections of fresh and preserved grafts before and 10 days after transplantation.*

Skin graft taking – a proteomic approach

A. Hegglin, N. Lindenblatt

The aim of this study was to identify differentially expressed proteins during skin graft taking with the goal to apply this knowledge to skin tissue engineering. The modified dorsal skinfold chamber was performed in B6 mice (n=30). Autologous full-thickness skin grafts were transplanted and harvested for proteome analysis after 0, 1, 3, 5 and 10 days.

Part of the identified proteins is assigned to the NO pathway: Arginase-1 was found to be decreased leading to an increase of active eNOS. Complementary to this, Ca2+ binding proteins (Sorcin, Parvalbumin, Tetranectin, Troponin T) showed a decreased expression, leading to increased level of Ca2+ ions required for eNOS activation.



Pooling of 6 skin samples per timepoint required due to low single sample weight (av. 20mg)

Fig. 3: Intravital microscopy of skin graft and wound bed reactions during taking and proteomic study approach

Each mouse underwent intravital microscopy to characterise the stadium of graft take and vascularisation *(Fig. 3:* 0d=normal skin, 1d=no perfusion, minor phenotypic angiogenic capillary changes, 3d=graft reperfusion, 5d= graft angiogenic response with bud formation, 10d= reestablishment of normal skin capillary pattern, no angiogenic changes*)*. Subsequently the protein fraction was separated in a 2D approach (4 gels/timepoint), followed by MS and MS-MS protein identification. This approach resulted in the identification of 52 differentially expressed proteins *(Fig. 4)*.

Other differentially expressed proteins (HSPB1, HSP6) belong to the group of heat-shock proteins, which are known to be involved in cell migration but, so far, were not yet described in the context of revascularisation and regeneration. Based on our first results, we conclude that the proteomic approach proved its suitability to deliver new insights into the process of skin graft taking on the protein level. Further analysis is on-going revealing the involvement of novel proteins in engraftment. This knowledge may be beneficial for tissue engineering of skin in the future.

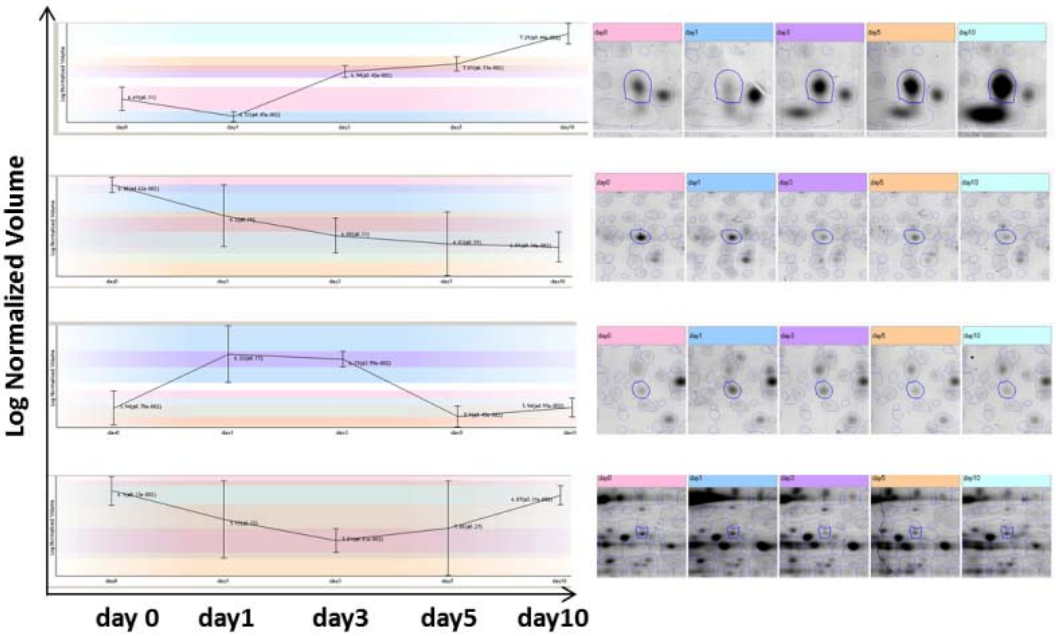


Fig. 4: Identification of diferentially expressed proteins bei MS and MS-MS

Collaborations/Sponsors:

- Prof. Dr. Simon P. Hoerstrup, MD, PhD, Department of Surgical Research and Clinic for Cardiovascular Surgery, UniversityHospital Zurich
- PD Dr. Ernst Reichmann, MD, Tissue Biology Research Unit, Children´s Hospital Zurich
- Dr. Christian A. Schmidt, MD, Clinic for Cardiovascular Surgery, UniversityHospital Zurich
- Ina Kalus, PhD, Rok Humer, PhD, Department of Internal Medicine, UniversityHospital Zurich
- Dr. Deon Bezuidenhout, MD, Cardiovascular Research Unit, University of Cape Town, South Africa
- Dr. Katrin Kerl, Department of Dermatology, UniversityHospital Zurich
- Prof. Dr. Urs Ziegler, Claudia Dumrese, Klaus Marquardt, Center for Microscopy and Image Analysis, University of Zurich
- Prof. Dr. Brigitte Vollmar, MD, Institute for Experimental Surgery, University of Rostock, Germany
- Prof. Dr. Martin Glocker, MD, Proteom Center, University of Rostock, Germany
- Prof. Dr. Michael D. Menger, MD, Institute for Clinical and Experimental Surgery, University of Saarland, Germany

2.5.4 Protein Profiling

Proteome signatures of free flaps - protein profile changes during ischemia and reperfusion

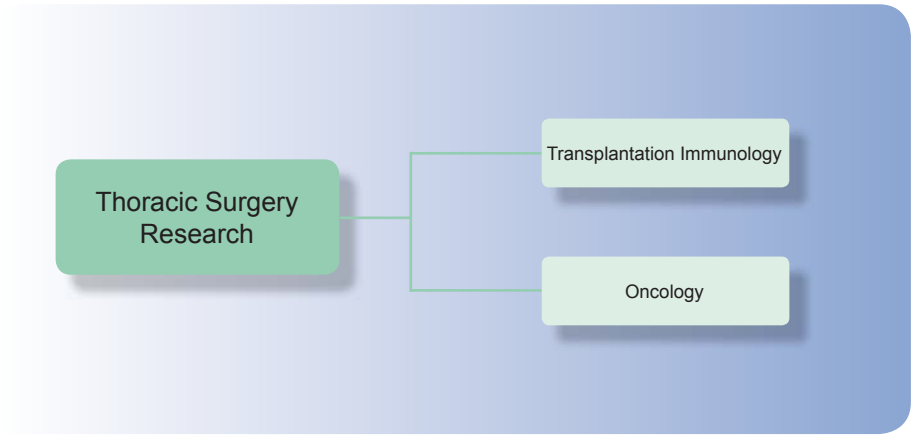
T. Lanaras, N. Lindenblatt, M. Bredell, M. Calcagni, P. Giovanoli

Transfer of free flaps has become the method of choice to cover and reconstruct major wounds and defects in plastic surgery. Monitoring ischemic processes on the molecular level will enable estimations on the individual transplant quality at a much earlier time, when clinical signs are not yet visible. The aim oft he project is to gain monitoring factors and possibly prognostic profiles for free flap healing and development. Plasma samples from three groups of transplants (osteocutaneous flaps, muscle flaps, and skinflaps) will be analyzed. In the future, this knowledge shall be transferred into an assay that will be made available to the surgeon who then will be enabled to optimize the transplantation process, i.e. to decide at an early time point - prior to the appearance of clinical signs of vascular problems - whether a free flap needs to be taken back for salvage procedure.

Collaborations/Sponsors:

- Dr. Juliane C. Finke, Klinik für Plastische und Ästhetische Chirurgie, HELIOS Klinikum Emil von Behring, Berlin, Germany
- Prof. Dr. med. Michael O. Glocker, Proteom Center Rostock, Universität Rostock, Rostock, Germany

2.6 Thoracic Surgery Research



PD Dr.
Sven Hillinger,
MD

Prof. Dr.
Walter Weder,
MD, Director

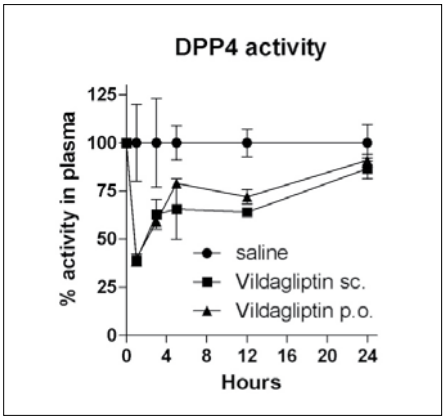
Prof. Dr.
Isabelle Opitz,
MD

2.6.1 Transplantation Immunology

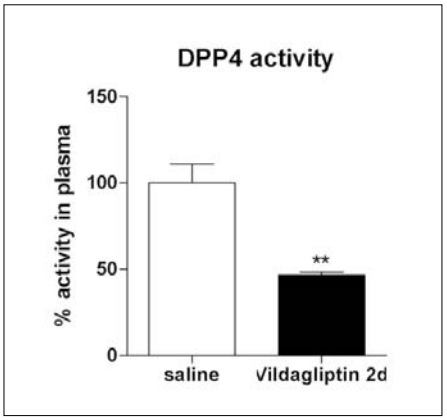
Improvement of ischemia-reperfusion injury in murine lung transplants by regenerative stem cells via inhibition of CD26/DPPIV

J.H. Jang, Y. Tsushima, I. De Meester, W. Weder, W. Jungraithmayr

Lung ischemia-reperfusion (I/R) - injury is a major cause of graft failure after lung transplantation (Tx). The glycoprotein CD26 that is abundantly expressed in pulmonary parenchyma, is a molecule that possesses a catalytic epitope (dipeptidyl peptidase IV, D PPIV), cleaving a host of key biologically active peptides including stromal cell derived factor 1α (SDF-1) which functions as a key modulator for stem cell homing in response to injury. Employing the physiological mouse model of orthotopic lung Tx, we found that Vildagliptin decreased DPP4 activity, enhanced SDF-1 levels, and decreased LDH levels , 2 days after I/R injury.



The kinetics of DPP4 activity by Vildagliptin administration measured in plasma



The activity of DPP4 inhibition by Vildagliptin, measured in plasma, 2 days of treatment (two times/day).

PD Dr.
Ilhan Inci,
MD

PD Dr.
Wolfgang
Jungraithmayr,
MD

Prof. Dr.
Stephan Korom,
MD

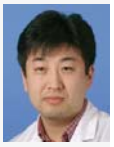
Dr.
Stephan Arni,
PhD

Dr.
Byron
Bitanihirwe,
PhD

Dr.
Stéphane
Collaud,
MD

Dr.
Mayura Meerang,
Postdoctoral
Fellow

Dr.
Thomas Wiedl,
PhD



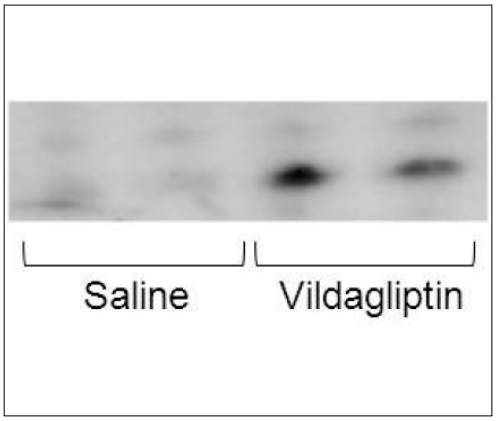
Dr.
Jae Hwi Jang,
PhD, Postdoctoral
Fellow

Dr.
Yukio Tsushima,
MD

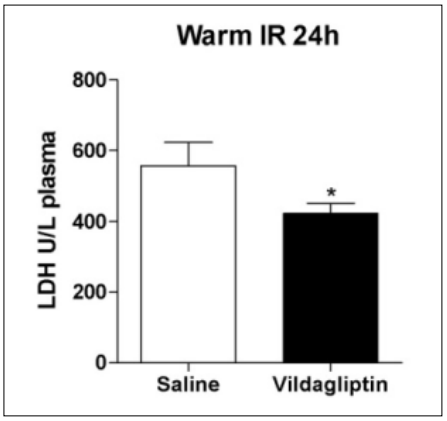
Nhung Le,
PhD Student

Manfred Welti,
Lab. Technician

Martina Friess,
Data Manager



Elevated SDF1 was detected by western blotting after two days of treatment.



I/R injury measured by plasma LDH levels, 24 hrs after reperfusion. Vildagliptin showed protective effect against ischemia/reperfusion injury..

Depletion of donor macrophages reduces graft damage and improves ischemia reperfusion injury in mouse lung transplants

Y. Tsushima, J.H. Jang, R. Schwendener, W. Weder, W. Jungraithmayr

Macrophages (M) are one of the most important cells of the innate immune system for first line defence. Chlodronate liposome successfully reduced 70% of macrophages from donor lungs when compared to grafts treated with empty liposome only. M-depleted transplants revealed significantly less graft damage and reduced HMGB1 when compared to control recipients. The inflammatory response (TNF-α) was significantly reduced in M-depleted mice when compared to control.

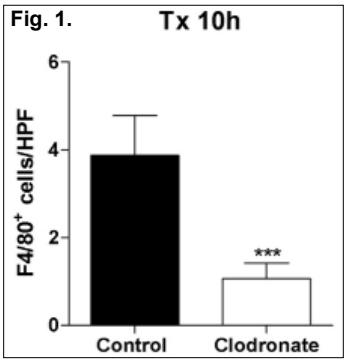


Fig. 1. Number of F4/80 pos. M in paraffin sections. Clodronate treated implant shows significantly less macrophages after Tx.

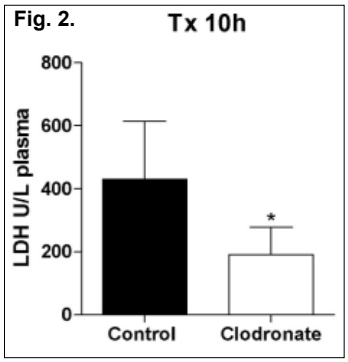


Fig. 2. Circulating HMGB1 levels, represents tissue injury, is reduced in Tx group with clodronate treated graft after ten hours of reperfusion.

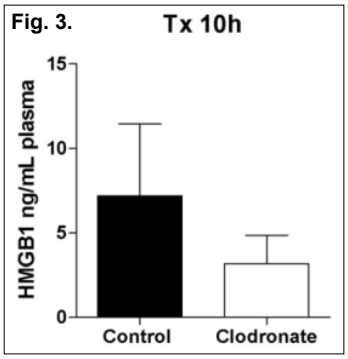


Fig. 3. The pro-inflammatory cytokine,TNFα is reduced in clodronate group compared to control group.

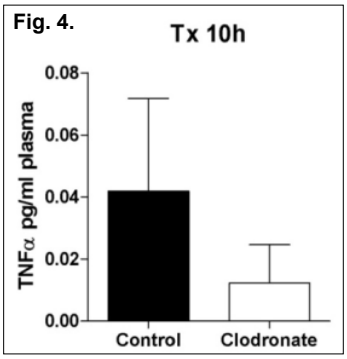


Fig. 4. Anti-inflammatory cytokine, TGFβ1 is elevated in clodronate group compared to control group.

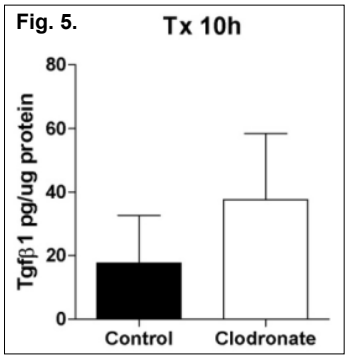


Fig. 5. Anti-inflammatory cytokine, TGFβ1 is elevated in clodronate group compared to control group.

Assessment of ischemia reperfusion injury by micro-computed tomography and ultra-short echo-time MRI after mouse lung transplantation

M. Wurnig, Y. Tsushima, W. Weder, W. Jungraithmayr, A. Boss

Ischemia/reperfusion-injury (I/R-injury) can severely impair the post-transplant course of lung transplant recipients. The purpose of this study was to compare ultra-short echo-time (UTE) sequences in magnetic resonance imaging (MRI) with a micro-computed-tomography (micro-CT) reference standard for the detection of I/R-injury in the experimental model of mouse single lung transplantation.

Comparing MR imaging and micro-CT, a similar diagnostic power in the detection of I/R-injury after lung transplantation could be observed. Due to complementary properties in the evaluation of dense and slight infiltration, the combination of both modalities seems favourable for the early detection of I/R-injury.

Collaborations:

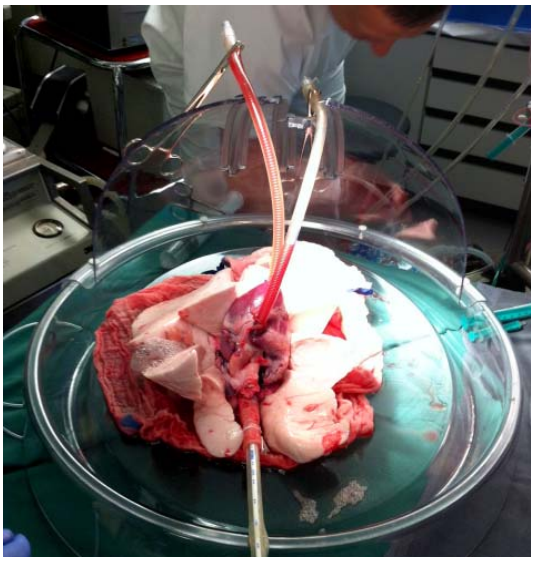
- Prof. Dr. Christian Münz, Prof. Dr. Burkhard Becher, Dr. Laura Codarri, (Institute of Experimental Immunology, University Irchel, Zurich)
- PD Dr. Dr. Andreas Boss, PD Dr. Thomas Frauenfelder, Dr. Natalie Chuck (Institute of Diagnostic Radiology, USZ)
- Dr. Johanna Buschmann (Klinik für Plastische Chirurgie und Handchirurgie, UniversitätsSpital Zürich)
- Prof. Dr. Ingrid de Meester, Dr. Veerle Mattheussen (Institute of Biochemistry, University of Antwerp, Belgium)
- Dr. Stefanie de Vleeschauwer (Institute of Pulmonary Research, University Leuven, Belgium)
- Dr. Ruedi Braun (Children Hospital, University of Madison, Wisconsin, USA)

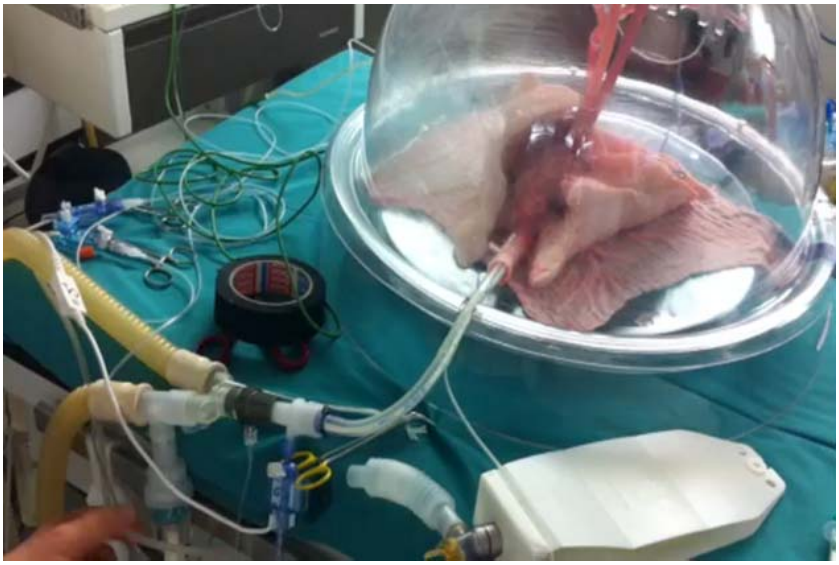
Ex vivo reconditioning of donor lungs with inhaled N-Acetyl cysteine after prolonged cold ischemia

I. Inci, S. Hillinger, S. Arni, T. Tsushima, W. Weder

Primary graft dysfunction continues to be an important limitation to a successful lung transplantation accounting for significant postoperative morbidity and mortality. Primary graft dysfunction is characterized by increased pulmonary vascular resistance, poor oxygenation, worsened compliance, and increased capillary permeability leading to edema formation. The ischemic insult during cold static preservation results in cytokine production and increased expression of adhesion molecules by hypoxic lung cells. The injury cascade is mediated mostly by neutrophil-endothelial adherence and subsequent neutrophil-mediated organ injury. Activated neutrophils secrete reactive oxygen species and proteolytic enzymes which results in structural and functional injury of the lung parenchyma.

N-Acetyl cysteine (NAC) is a precursor of the most important physiological antioxidant glutathione and a sulfhydryl containing substance. Sulfhydryl-containing compounds, especially glutathione, are important in the protection of cells





against hydroperoxide damage. This important reducing agent and antioxidant is involved in maintaining the cellular oxidation-reduction balance, and has been shown to protect cells from a wide variety of endogenous and exogenous insults.
The *ex vivo* lung perfusion (EVLP) is as a method, not only to evaluate lungs before transplantation, but also recondition lungs of inferior quality outside the body. The system comprises a centrifugal pump, a cardiopulmonary bypass membrane oxygenator supplied with 91% nitrogen and 9% carbon dioxide gas serving as a de-oxygenator, leukocyte filter, a venous reservoir, a heat exchanger and polyethylene tubing.
Recently we have shown that using rat and pig left single lung transplantation model, both donor and recipient treatment with NAC resulted in significant improvement of graft oxygenation, preservation of lung tissue reduced glutathione levels, decreased lipid peroxidation and peak airway pressures after prolonged cold storage.

In this experimental study we want to investigate whether donor lung reconditioning with inhaled NAC in EVLP system after 24 hours of cold ischemic storage would reduce primary graft dysfunction following lung transplantation. Therefore, we design the experiment to investigate the role of inhaled NAC in EVLP system after prolonged cold ischemic storage.
The donor lung block will be retrieved and stored for 24 hours at 4 degrees Celsius. The lung graft will be evaluated and reconditioned in EVLP system and then the left lung will be transplanted. In the control group (N=6) lung transplantation will be performed without any treatment, in the treatment group (N=6) donor lung will be reconditioned with inhaled NAC (150 mg/kg) in the EVLP system. After transplantation the observation time will be 6 hours.
We expect to have improved graft function in the reconditioned group with inhaled NAC compared to controls.

2.6.2 Oncology

2.6.2.1 Lung Cancer

Selective reaction monitoring for activity-based proteomics in human lung cancer biopsies

N. Le, M. Matondo, S. Hillinger, T. Wiedl, W. Weder, R. Aebersold, S. Arni

Our long-term goal is the use of the activity profile of the serine hydrolase (SH) enzyme superfamily as potential biomarker candidates in human lung adenocarcinoma (LA). In the discovery phase, we could demonstrate that the SH activities of ESD and ABHD11 as potential biomarker candidates for LA, both in a statistically significant model. Now we validate ABHD11, ESD and other SHs activity profiles with a larger cohort of patient tissues and with the selected reaction monitoring (SRM) methodology.

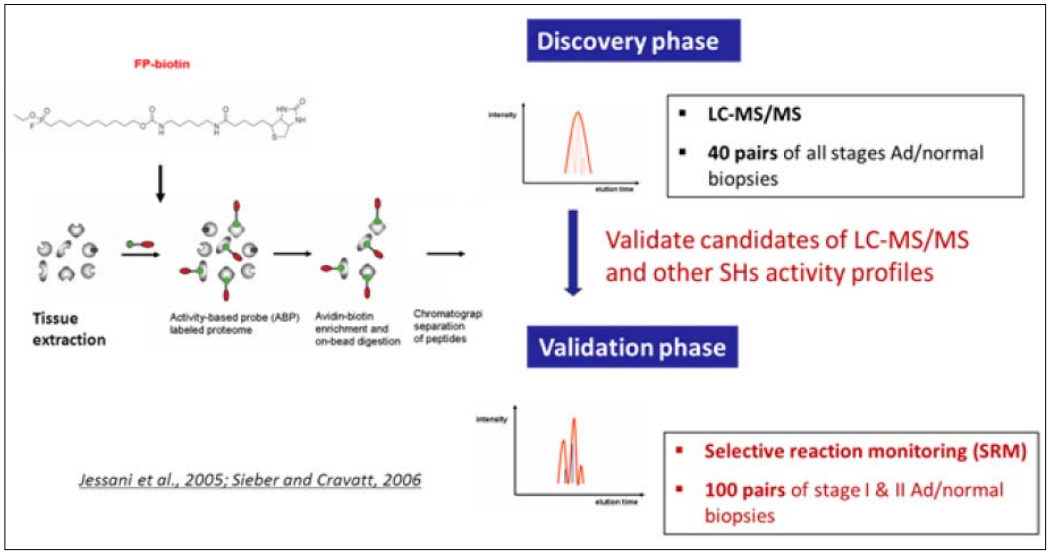


Fig.1. The discovery phase and validation phase of our studies

From the list of 121 serine hydrolase enzymes defined by our previous LC-MS/MS, other publications and databases, we detected 92 SH enzymes by SRM experiments, including our 2 candidates ESD and ABDH11.
We anticipate that ESD and / or ABHD11 activities have the potential to develop into molecular predictors with a reliable clinical significance. The implemented activity-based proteomics platform represents a powerful tool in the search for novel disease biomarkers.

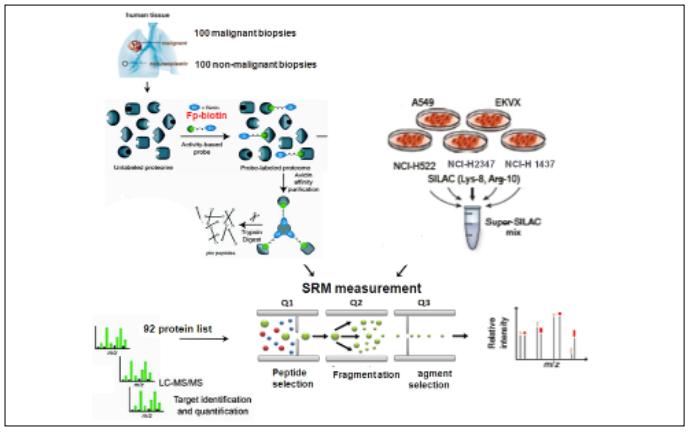


Fig.2. Workflow of SRM-based proteomic experiments for the validation phase

Multiplex profiling of protein tyrosine kinase substrates in early stages human lung adenocarcinoma

S. Arni, T. Hong, N. Le, W. Weder, S. Hillinger

Biomarkers with reliable prognostic significance are of utmost importance but due to a lack of immediate correlation between levels of protein and their corresponding mRNA, a screen based on the kinase activity become a promising option to circumvent this limitation with the tremendous advantage of focusing on therapeutically targetable enzymatic activities. We screened 74 paired malignant TNM stage 1 and 2 lung adenocarcinoma and non-neoplastic lung biopsies for the multiplex tyrosine phosphorylation of substrates immobilized on a PamChip®4microarrays. Based on a 76-point 'response-signature' we obtained 73 % of correct prediction with a 10 fold cross validation PLS-DA analysis in TNM stage 1 lung adenocarcinoma biopsies. Moreover, we detected 26 peptides substrates significantly more inhibited in kinomes of long-term survivors than in kinomes of the short-term survivors. Furthermore, the found differences in enzymatic activities in lung biopsies may result in the identification of new targets in future anti lung cancer therapy efforts.

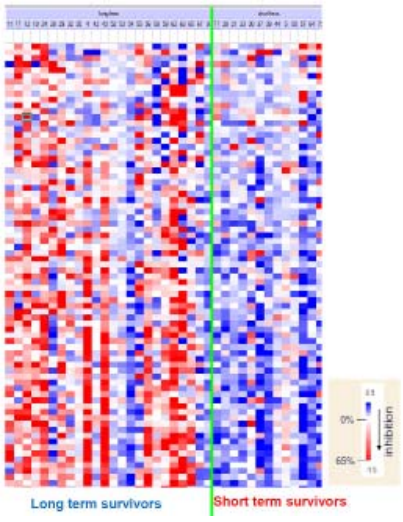


Figure 1
Heatmap showing fold change of peptide tyrosine phosphorylations for TNM stage 1 lung adenocarcinoma patients sorted according to survival . The Log2-transformed ratio of phosphorylation for the biopsies of malignant versus malignant treated with the PTKI gefitinib of the 76 selected peptides used for the signature are sorted according to correlation with patient survival. Inhibition is scaled per peptide and the red color indicate higher inhibition of phosphorylation. The vertical green bar indicates the separation of the long-term survivors in the left side from the short-term survivors in the right side.

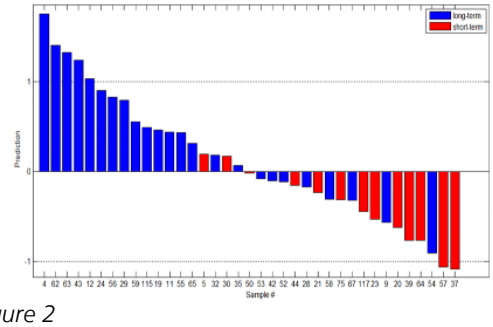


Figure 2
TNM stage 1 lung adenocarcinoma PLS-DA class prediction result performed with the 76 selected peptides. Prediction performance was tested using 10-fold cross-validation for 37 samples tested to obtain here 73% correct prediction. Prediction performance is greater than zero the samples are allocated to the long-term survivors groups or to the short-term survivors when prediction performance smaller than zero. Prediction performance which is further away from the decision boundary set at 0 is less likely to really belong to the opposite group.

Collaborations:

- Prof. Dr. Ruedi Aebersold, Dr. Mariette Matondo (Institute of Molecular Systems Biology, Swiss Institute of Technology, Zurich)
- Dr. Rik de Wijn, Dr. Martjin Dankers (PamGene International, 's-Hertogenbosch, The Netherlands)
- Prof. H. Moch and Dr. A. Soltermann, Department of Pathology, UniversityHospital Zurich

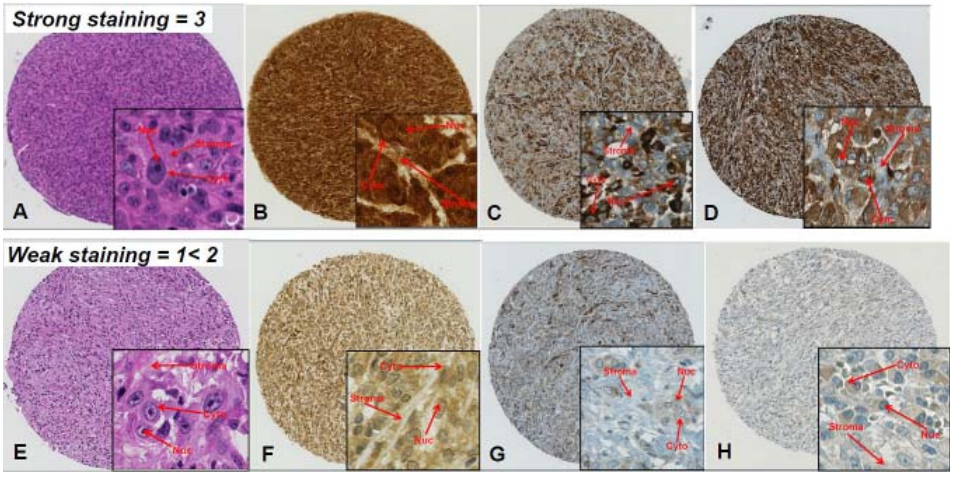
2.6.2.2 Malignant pleural mesothelioma

Clinically relevant prognostic markers for malignant pleural mesothelioma: The significance of the PI3K/mTOR signaling Pathway

B. Bitanihirwe, I. Opitz

Malignant pleural mesothelioma (MPM) is a rare and aggressive tumor of mesothelial cell origin that is strongly associated with previous asbestos exposure. The prognosis for MPM is dismal, with a median survival of less than 12 months without treatment. Against this dismal background, prognostic indicators that may assist in understanding the molecular pathogenesis of MPM and the mechanisms that might underlie resistance of this malignancy to current therapy are needed to more accurately determine prognosis.

The phoshoinositide-3 kinase (PI3K)/mammalian target of rapamycin (mTOR) pathway is probably one of the most important pathways in cancer metabolism and growth. A set of pre- and post-induction chemotherapy biopsies from patients diagnosed with MPM uniformly treated within a multimodal context (viz. induction chemotherapy and extrapleural pneumonectomy) were employed in our study. Expression levels of markers of PI3K/mTOR pathway are analyzed.



Immunohistochemical staining of PTEN, p-mTOR and p-S6 in MPM in an epitheloid subtype. H&E staining of respective MPM cores (A and E). Cytoplasmic and nuclear PTEN expression (B - Strong and F - Weak). Cytoplasmic expression of p-S6 (C - Strong and G- Weak). Cytoplasmic expression of p-mTOR (D-Strong and H- Weak). Abbreviations: Cyto = Cytoplasm; Nuc = Nucleus.

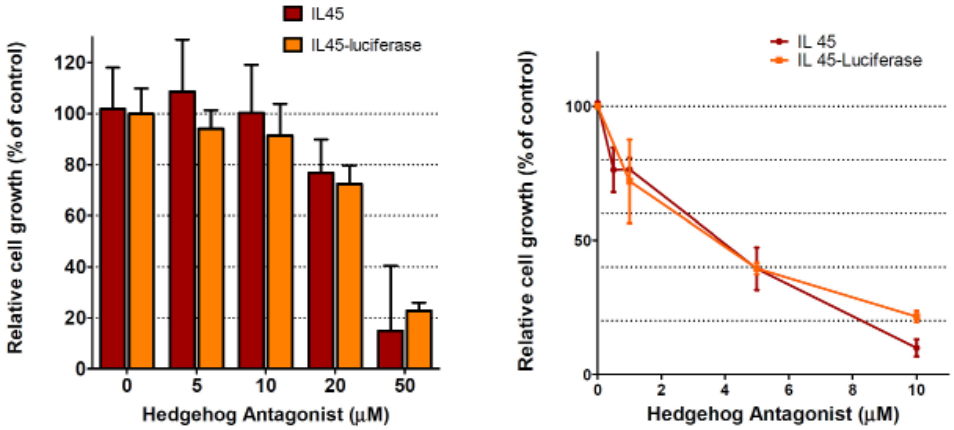
Collaborations:

- Toronto General Hospital, University of Toronto (Dr. Ghassan Allo, Dr. Ming Tsao, Prof. Dr. Marc de Perrot)
- Biostatistics Unit, University of Zurich (Prof. Dr. Burkhardt Seifert)
- Laboratory of Molecular Oncology, USZ (Prof. Dr. Rolf Stahel, PD Dr. Emanuela Felley-Bosco)
- Department of Pathology, USZ (Prof. Dr. Holger Moch, Dr. Alex Soltermann, Dr. Bart Vrugt, Dr. Svenja Thies and Lukas Frischknecht)
- Institute of Molecular Medicine, St. James's Hospital, Dublin (Dr. Steven Gray)

Investigation of the role of hedgehog signaling on malignant pleural mesothelioma recurrence by intracavitary treatment with a hedgehog inhibitor

M. Meerang and I. Opitz

Multimodal treatment currently provides the best survival outcome for malignant pleural mesothelioma (MPM). However, local tumor recurrence remains a significant challenge. A chemoresistant side population of cells which retain precursor properties has recently been identified in MPM *(Frei, Opitz et al. 2011)*. Because stem cell activation through the hedgehog signaling pathway could be detected in the side population as well as in MPM biopsies *(Shi, Moura et al, 2012)* we analyze the *in vitro* and *vivo* role of hedgehog activation for MPM recurrence. A hedgehog antagonist will be assessed in our MPM recurrence model.



Hh antagonist dose-dependently suppressed growth of mesothelioma cell lines.
MTT (a) and colony formation assay (b) showing that IL45 are sensitive to Vismodegib. Identical growth suppression of IL45 and IL45 luciferase by Vismodegib confirmed that luciferase expression does not influence IL45 properties.

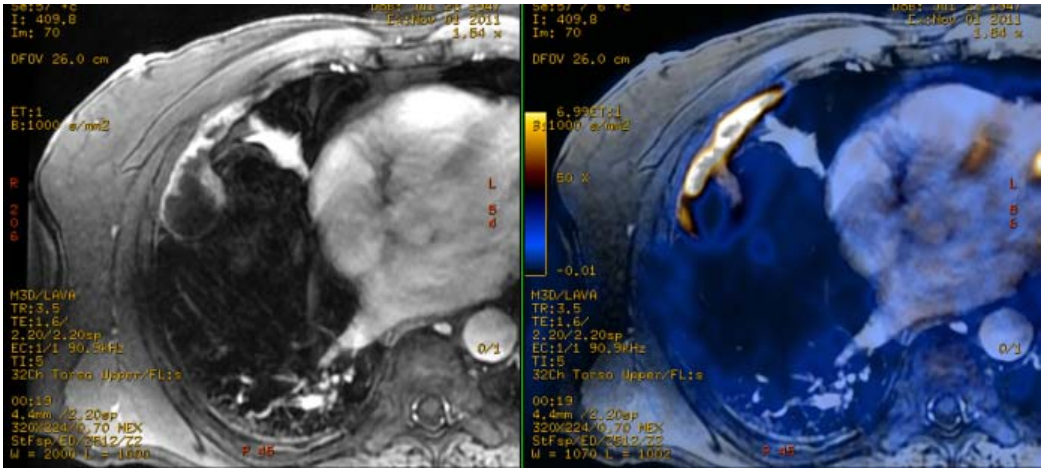
Collaborations:
- Laboratory of Molecular Oncology, USZ (Prof. Dr. Rolf Stahel, PD Dr. Emanuela Felley-Bosco)

Clinical Staging for malignant pleural mesothelioma

I. Opitz, W. Weder

In this project, we aim to assess the value of different modalities for a better clinical staging and assessment of therapy response of malignant pleural mesothelioma (MPM) including computed tomography (CT)-scan, positron emission tomography-CT (PET-CT), PET-magnetic resonance (MR) *(Frauenfelder 2011)*.

Patients with proven MPM will undergo chest CT scan and PET-CT before and after chemotherapy. Tumor response will be measured and classified with modified RECIST criteria and compared to tumor volumetric approach in CT-scans, the metabolic response will be defined in PET-CT.



Addition of MRI improves detection of diaphragmatic and chest wall invasion (Heelan et al. 1999)

Collaborations:
- Laboratory of Molecular Oncology, USZ (Prof. Dr. Rolf Stahel)
- Institute of Biostatistics, UZH (Prof. Dr. Burkhardt Seifert)
- Institute of Radiology, USZ (Dr. Thomas Frauenfelder, Dr. Roger Hunziker, Dr. Thi Dan Linh Nguyen-Kim)
- Division of Nuclear Medicine, USZ (PD Dr. Patrick Veit-Haibach, PD Dr. Lars Husmann)

Localized intracavitary therapy for MPM – from bench to bedside

I. Opitz, W. Weder

Our newly developed intracavitary chemotherapy with cisplatin loaded in to a fibrin carrier for MPM will be applied into clinical application *(Lardinois, Jung et al. 2006; Opitz, Lardinois et al. 2007; Opitz, Erne et al. 2011)*. Safety and tolerability, and later efficacy will be assessed in mesothelioma patients who underwent prior surgery (Phase I Dose-Escalation and Phase IIa Monocentric Open Trial for the Evaluation of the Safety of ***In***tracavitary Cisplatin-***F***ibrin ***L***ocalized ***C***hemotherapy after Mesothelioma surgery for the Treatment of Patients with Malignant Pleural ***Mes***othelioma, ***INFLuenCe – Meso***).

Collaborations:

- Division of Pharmacology and Toxicology, UniversityHospital Zurich (Dr. Alexander Jetter)
- Laboratory of Organic Chemistry, Swiss Federal Institute of Technology (Prof. Dr. Detlef Günther)
- Laboratory of Molecular Oncology, UniversityHospital Zurich (Prof. Dr. Rolf Stahel, PD Dr. Emanuela Felley-Bosco)
- Guillaume Wuilleret (Dissertation)

In vivo study of the efficiacy of a dual phosphatidylinositol-3-kinase (PI3K)-/mTOR inhibitor in the treatment of malignant pleural mesothelioma

S. Tomaszek, O. Lauk, I. Opitz, W. Weder

The efficacy of novel agents such as phosphatidylinositol-3-kinase (PI3K)/mTOR-inhibitor was studied successfully *in vitro* and *in vivo* in various solid tumors, resulting in clinical phase I/II studies (currently accruing).

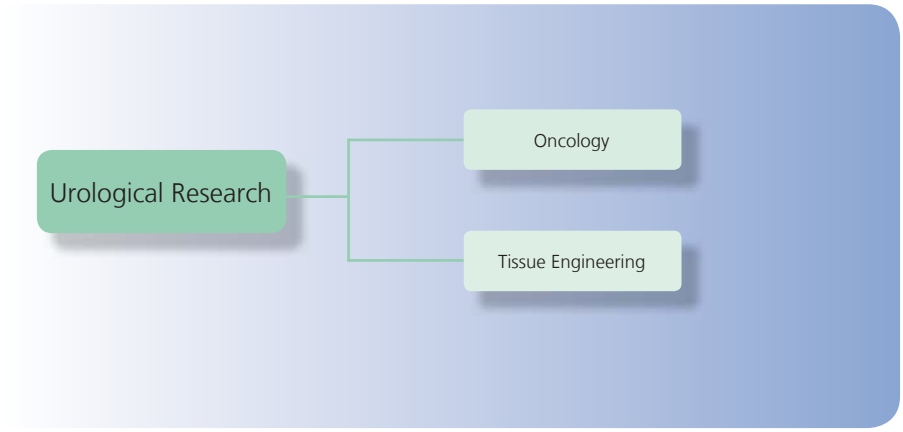
However to date, there are no data available on the efficacy of dual PI3K/mTOR-inhibitors in malignant pleural mesothelioma. Studies with the selective mTOR inhibitor Temirolimus in malignant pleural mesothelioma *in vitro* have shown a potent inhibition of mTOR-induced signals resulting in a cytostatic effect in human cell lines. This was increased in combination with simultaneous treatment with cisplatin. These observations were reproducible in an *in vivo* xenograft model.

The advantage of the dual PI3K/mTOR-inhibitor NVP-BEZ235 compared to conventional mTOR-inhibiting agents is the inhibition of PI3K as well as mTOR and hence various effectors of the signaling pathway, which may result in superior efficacy as well as improved drug compatibility. In the present study this inhibitor will be tested in a mouse model.

Collaborations:

- PD Dr. Emanuela Felley-Bosco and Prof. Dr. Rolf Stahel, Laboratory of Medical Oncology, UniversityHospital Zurich
- Prof. Dr. D. Fennell, Belfast Cancer Research UK Centre, Belfast, UK
- Prof. Dr. R. Stahel, PD Dr. E. Felley-Bosco, Labor für Molekulare Onkologie, UniversitätsSpital Zürich
- Dr. S. Gray, Translational Cancer research Group, Trinity Centre for Health Sciences, Institute of Molecular Medicine, St. James's Hospital, Dublin, Ireland
- Prof. Dr. H. Moch, PD Dr. A. Soltermann, Dr. B. Vrugt, Institut für klinische Pathologie, UniversitätsSpital Zürich
- Dr. M. de Perrot, Division of Thoracic Surgery, Toronto General Hospital and Princess Margaret Hospital, University of Toronto, Toronto, Canada

2.7 Urological Research



PD Dr.
Maurizio
Provenzano,
MD, PhD

Prof. Dr.
Tullio Sulser,
MD, Director

PD Dr.
Daniel Eberli ,
MD, PhD

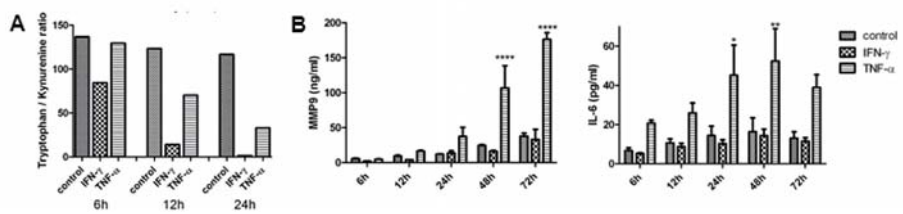
Damina Balmer,
Scientific
Coordination

2.7.1 Oncology

The role of pro-inflammatory stimuli in modulating the expression and function of prostate cancer-released tumor derived factors

I. Banzola, C. Poyet, B. Fischer, D. Eberli, T. Sulser, M. Provenzano

Histological analysis of prostatic samples has revealed that in the areas surrounding adenocarcinomas, inflammation is often detected in lesions designated as early stages of the disease. We are indeed studying how inflammatory cytokines, such as IFN-γ and TNF-α, could trigger the release of prostate cancer related tumor derived soluble factors (PCa-TDSFs) and how this elicitation might affect the cancer microenvironment. Among TDSFs, our interest focused on those microenvironmental modifiers that have been reported as possible mediators of PCa morbidity, such as 2,3-indolemanine deoxygenase (IDO), interleukin-6 (IL-6), matrix metalloprotein 9 (MMP9), transforming growth factor-beta (TGF-β).



IDO activity (Panel A) and protein release (MMP9 and IL-6) (Panel B) in PCa cells after cytokine stimulation

Dr.
Souzan Salemi,
PhD

Dr.
Cédric Poyet,
MD

Dr.
Boris Fischer,
MD

Dr.
Maya Horst,
MD

Dr.
Remo Largo,
MD

Dr.
Meline Stölting,
MD, PhD Student

Dr.
Fahd Azzabi,
PhD

Dr.
Lukas Brügger,
MD

Dr.
Roman Inglin,
MD


Irina Banzola, PhD


Sarah Nötzli, M.Sc.

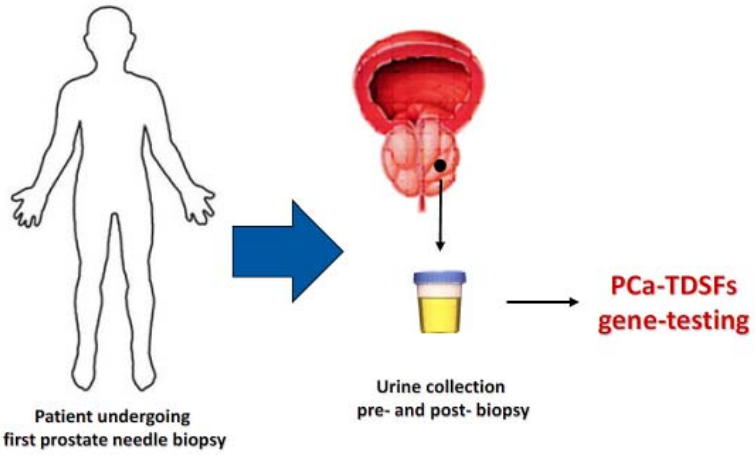

Deana Haralampieva, PhD Student


Ria Tauscher, Master Student

Multiplexed gene expression of indoleamine 2,3-dioxigenase and prostate cancer related tumor derived soluble factors (PCa-TDSFs) in urine of patients undergoing first prostate needle biopsy

B. Fischer, I. Banzola, C. Poyet, T. Sulser, M. Provenzano

The search for biomarkers to precisely and non-invasively characterize the biology of prostate cancer (PCa) is the focus of many laboratories across the world. So far, a huge amount of biomarkers are under consideration for PCa diagnosis, prognosis and therapeutic interventions, but validation studies are needed to upgrade them to clinically useful markers. Here, we propose a promising approach integrating multiplexed gene analysis for the simultaneous test of several prostate cancer related markers, so called tumor derived soluble factors (PCa-TDSF).

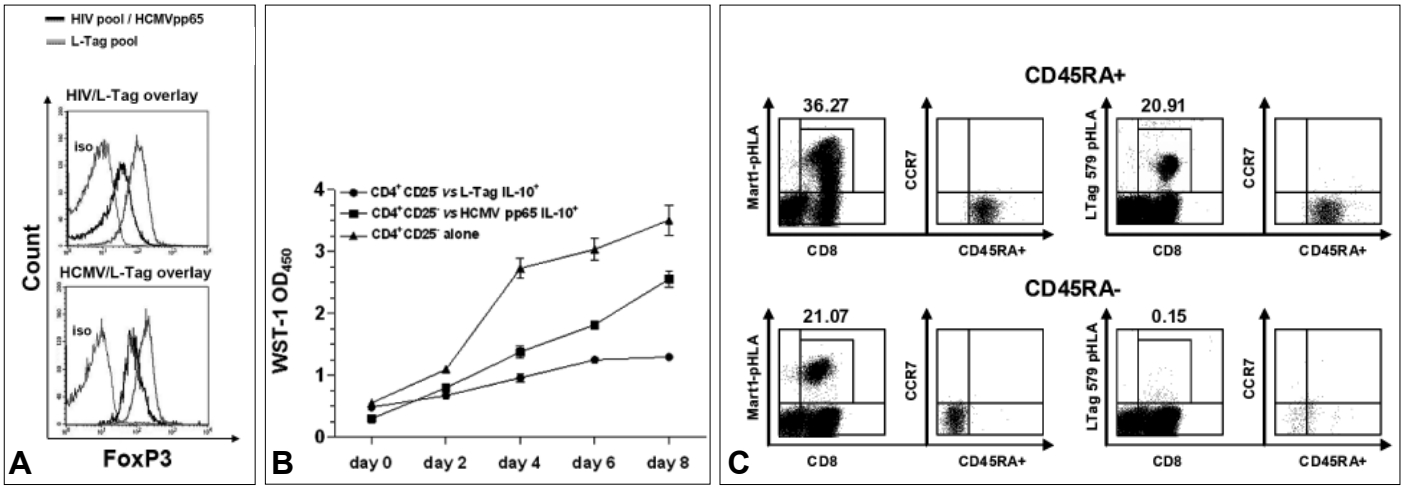


Study design for urine collection

Polyomavirus BK large tumor antigen exerts tolerogenic signatures with immunodominant p53-binding regions in prostate cancer

G. Sais, I. Banzola, H.H. Hirsch, T. Sulser, M. Provenzano

Polyomavirus BK (BKV) large tumor antigen (L-Tag) has been recently identified as a potential co-factor in the development of prostate cancer (PCa) but its role as target of immune responses in this malignancy remains unexplored. A regulatory profiling (IL-10 and TGF-β-producing $CD4^+CD25^+FoxP3^+$T cells) elicited by L-Tag has been observed in BKV seropositive PCa patients while T cells against p53-binding regions of same antigen exquisitely belong to effector/memory CD8+ T cells populations in BKV seropositive healthy donors. The ambiguous activity of L-Tag prompted us to investigate the role of functional regions within L-Tag in eliciting peculiar immune responses in PCa.



*BKV L-Tag-specific CD4+ T cells with CD25+, FoxP3+ regulatory phenotype in L-Tag IgG+ patients with BKV+ PCa. (A) FoxP3 MFI upon L-Tag, HIV peptide pool and HCMVpp65$_{340-355}$ peptide stimulation; (B) Proliferation index of anti-CD3/CD28 stimulated CD4+CD25-Tcells after cocultures with autologous L-Tag-specific IL-10+ CD4+CD25+CD127- Tcells; (C) Phenotype of CD8+Tcells from BKV seropositive healthy donors stained with either MelanA/Mart-1$_{26-35}$ or BKV LTag$_{579}$ specific HLA-A*0201 multimers after a 3-week peptide* in vitro *stimulation*

The oncogenic polyomavirus BK large tumor antigen (L-Tag) as potential marker for prognosis in prostate cancer

M. Provenzano, H.H. Hirsch, T. Sulser

We have recently observed a dramatic correlation between the detection of BKV L-Tag in PCa lesions (BKV+) and the evidence of biochemical recurrence (BR+) in patients bearing these lesions. Although data do not allow the postulation of any causal relationship between BKV infection and PCa, they undoubtedly suggest for a putative role of BKV L-Tag in PCa progression. Indeed, a more robust investigation on the detection of BKV L-Tag in prostate cancer lesions could unravel whether this antigen could be a target in the progression of prostate cancer and hopefully be upgraded to a potential clinically useful biomarker for this disease.

Association between BR status and BKV+ Pca

	BKV+	BKV-	
BR+	7	0	7
BR-	8	17	26
	15	17	32

• **Fisher exact test, p=0.004**

TABLE 3

Analysis of L-Tag IgG$^+$ patients bearing BKV$^+$ PCa with or without BR[a]

Patient[b]	BR (PSA ng/ml)[c]	L-Tag DNA (copies/10^5 cells)	L-Tag IgG (OD_{492})
IgG$^+$ BKV$^+$ BR$^+$** PCa (n = 7)			
PCa 2	6	NT	+
PCa 8	6	+	+
PCa 18	6	+	+
PCa 27	96	+	+
PCa 37	6	+	+
PCa 48	48	+	+
PCa 49	24	+	+
PCa 57	6	+	+
PCa 60	6	NT	+

• **Time of BR in weeks**

Bladder cancer microenvironment and maturation signature of lymphatic endothelial cells

C. Poyet, I. Banzola, T. Sulser, M. Provenzano

Approximately 25% of patients undergoing surgery for bladder cancer show lymph nodal metastases (LN+). Indeed, there is a high interest in studying the role of lymph-angiogenic factors in patients bearing superficial cancers (NMIBC) with high risk of disease progression (LN+), in order to redirect treatment options. Our aim is thus tempting to speculate that the generation of a new lymphatic vasculature, mainly sprouting from pre-existing blood vessels, could also occur from pre-existing lymphatic vessels, a possible event in cancer microenvironment.

Collaborations:

- Institute for Medical Microbiology and Division of Infectious Diseases, University of Basel.
- Institute for Surgical Research and Hospital Management, Oncology section, University Hospital of Basel.
- Institute of Pharmaceutical Sciences, ETH, Zurich.

2.7.2 Tissue Engineering for Urologic Tissue

Tracking of human Muscle Precursor Cells by MRI and muscle regeneration *in vivo*

F. Azzabi Zouraq, V. Jovaisaite, L. Hefermehl, A. Boss, M. Rudin, T. Sulser, D. Eberli

Stress Urinary Incontinence (SUI), the involuntary loss of urine, is a medical problem that affects millions of people worldwide. Recent research suggests stem cell therapy as a potential solution to restore a normal sphincter function. Clinical trials to treat SUI by autologous Muscle Precursor Cells (MPC) transplantation are currently in preparation. In addition to functional follow-up, evaluation of cell survival and tissue formation is essential for understanding and improvement of cell therapies. In this study we explored detrimental effects of increasing intracellular levels of SPIO *in vitro* and aimed to define a safe concentration in which human MPCs can be easily detected by MRI, without altering their cellular functions.

Using SPIO concentration of 400µg/mL, no effects on cell viability, growth, differentiation and muscle - specific marker expression were detected, while labelling allowed the cells to be detected by MRI. Labelled human MPCs were then injected into the subcutaneous space of nude mice. Transplanted cells formed a muscle tissue, as confirmed by histological analysis.

Location of the muscle was detectable by MRI for at least 4 weeks. Our data concludes that the optimized conditions of MPC labelling can be safely used in clinics to track sphincter muscle regeneration in patients under SUI treatment by cell therapy.

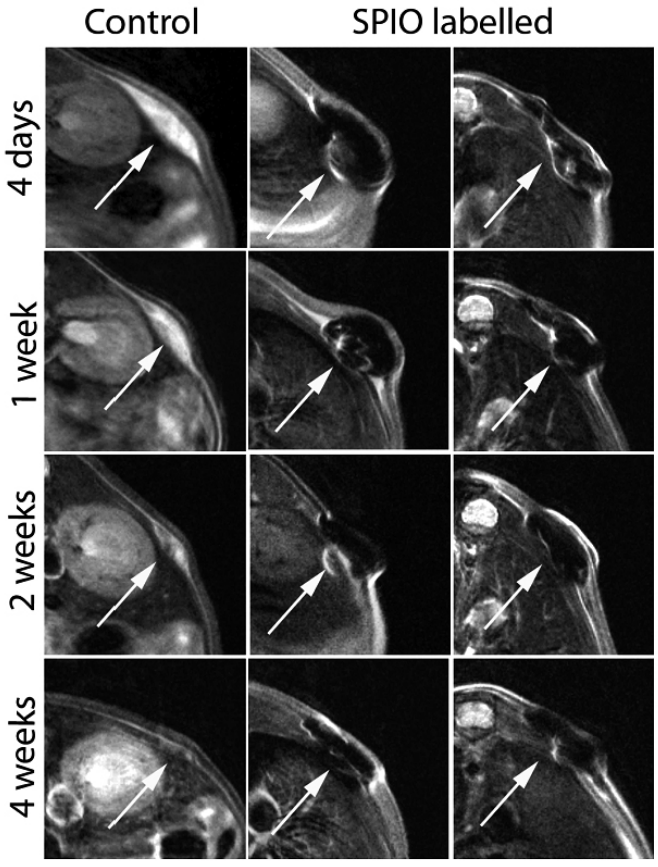
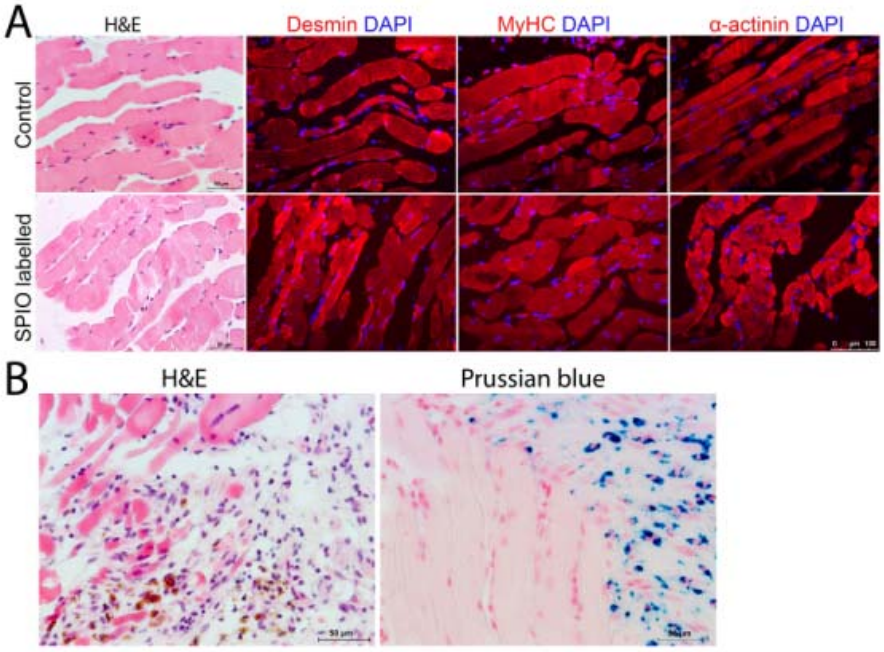


Figure 1: MRI scanning of injected human MPCs without and with iron oxide particles (control). Cultured MPCs from biopsy were labelled with iron oxide particles (SPIO) one day prior to injection subcutaneously in nude mice. The cells could be tracked for as far as for 4 weeks by MRI scanning.

Figure 2: Fiber formation and immunohistochemistry of tissue engineered muscle after MPCs injection in nude mice. Human cultured MPCs and labelled with iron oxide particles were injected subcutaneously in nude mice. After 4 weeks, the tissue engineered muscle were harvested and stained with different technics (H&E, Prussian blue and specific marker of muscle as desmin, Myosin heavy chain and alpha-actinin). The injected SPIO labelled MPCs were capable in forming fibers and possess specific characterisation of muscles.

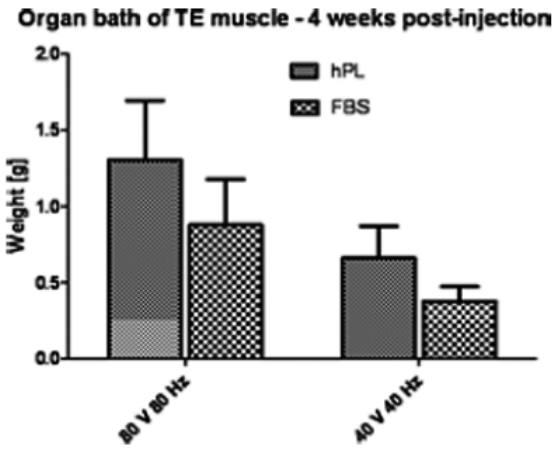


Xeno-free culturing of human Muscle Precursor Cells (MPC) for clinical application

F. Azzabi Zouraq, R. Tauscher, S. Salemi, R.A. Largo , K. Schallmoser, D. Strunk, T. Sulser, D. Eberli

Autologous cell therapies are envisioned as a promising therapy for many diseases including urinary incontinence, where a sphincter muscle damage leads to urine leakage and significantly reduces the quality of life. Recent animal models demonstrated the validity of the method showing regeneration of sphincter muscle tissue after injection of MPCs. Currently, these cells are expanded in xenogenic media containing fetal bovine serum (FBS). However, before MPCs can be applied clinically it is mandatory to reduce the potential immunogenic reaction and infection risk by removing any xenogenic contaminants.

In this research human MPCs were expanded in xeno-free medium using pooled human platelet lysates (phPL) or pooled human Serum (HS). Our results show that hPL is a suitable substitute for FBS and may be used for clinical application.



Organ bath of formed tissue engineered muscle. Injected MPCs grown in different mediums based either on FBS or pooled human platelet lysate were injected subcutaneously in nude mice. Four weeks post-injection, the tissue engineered muscle were harvested and tested for their contraction with organ bath technics. The tissue engineered muscles rising from cells grown in xeno-free mediums were also capable in giving functional muscles.

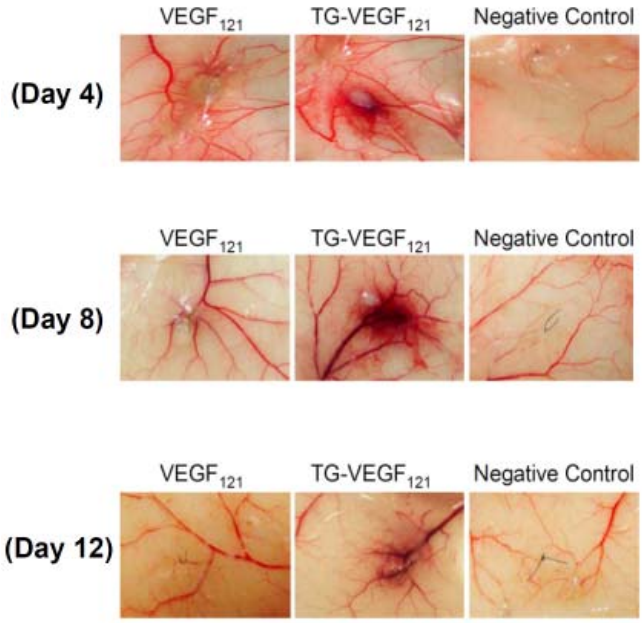
Induction of Mature Vascular Networks *in vivo* **by Longterm Cell-demanded Release of TG-VEGF121**

R. Largo, J. Marschall, V. Ramakrishnan, A. Ziogas, J. Plock, T. Sulser, J. Hubbell, K. Lorentz, D. Eberli, M. Ehrbar

The establishment of mature vascularisation in ischemic and engineered tissues by biomaterial engineering and growth factor delivery remains a major challenge. To modulate the proteolytic stability of fibrin gels and consequently the release profile of the VEGF we have simultaneously incorporated TG-VEGF and protease inhibitor Aprotinin (TG-Aprotinin). Fibrin gels that were formed in presence or absence of TG-VEGF and contained different concentrations of TG-Aprotinin were subcutaneously implanted in the back of immunocompromized mice. Our gross morphological, histological, and histomorphometrical evaluations indicate that vascular leakiness and angiogenic response (vessel number and maturity) is greatly dependent on the fibrin gel stability. This translational work not only represents a significant step forward towards developing mature vascular networks for engineered tissues but might also find use in other tissue engineering and drug delivery applications.

Improving Urinary Sphincter Engineering by Pre-Establishment of a Vascular Network

R. Largo, V. Ramakrishnan, J. Marschall, A. Ziogas, J. Plock, T. Sulser, M. Ehrbar, D. Eberli



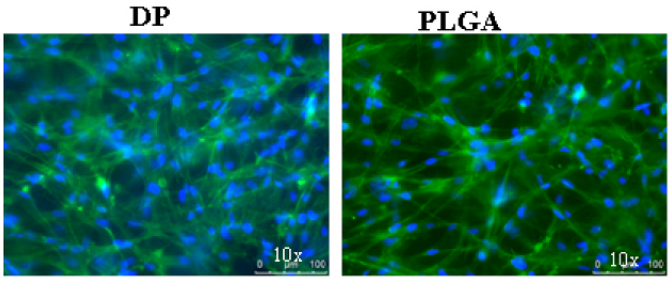
The use of autologous cells could be ideal in reversing sphincter muscle damage. Until now, the construction of large volumes of functional muscle tissue with muscle precursor cells (MPC) is limited by insufficient angiogenic induction. We engineered a fibrin gel which allows cell-demanded release of covalently bound TG-$VEGF_{121}$ (TGV). Our *in vitro* data demonstrated the stability (confirmed by ELISA) and maintenance of biological activity of TGV over 3 weeks, which was confirmed by HUVEC proliferation assay and Western blot of proteins involved in downstream signalling pathways (pVEGF-R, ERK1/2). Our data demonstrate that fibrin constructs containing covalently-bound TGV can form a robust neo-vascular network. Within a translational context, this slow release material can be injected directly into a damaged sphincter before cell therapy. Well-vascularized tissue could support functional muscle tissue development after cell injection. This method could impact many organ systems and help to overcome current limits in organ engineering.

Use of Adipose Derived Stem Cells for Bladder Engineering

S. Salemi , R. Gobet, T. Sulser, D. Eberli

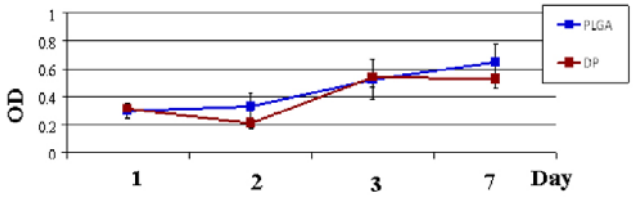
Adipose derived stem cells (ADSCs) can be differentiated to smooth muscle cells (SMCs) and might offer a cell source for hollow organ engineering. However, differentiation is a complex process and has a dramatic effect on cell size, shape, membrane potential, metabolic activity and responsiveness to external signals.

WB analysis revealed increased levels of smoothelin and calponin protein expression during differentiation process. Seeded ADSCs on PLGA and DP adhered very well on the scaffolds and an increased proliferation was observed.



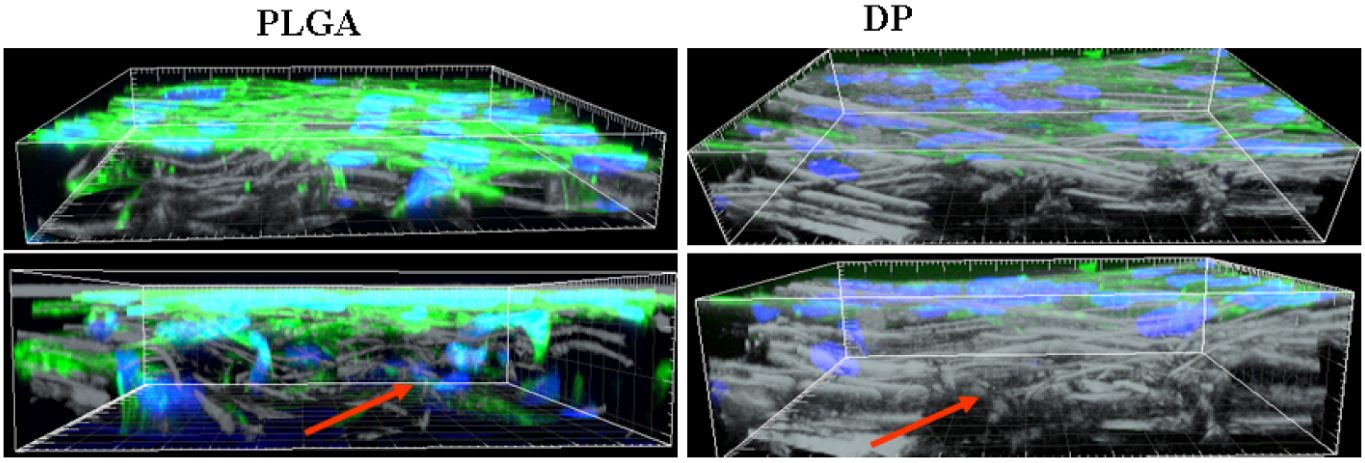
A

Immunostaining of ADSCs
ADSCs were cultured on DP and PLGA for 72h. Green colour indicates cell growth and proliferation of ADSCs within the scaffold. Green; Phalloidin staining for cytoplasm. Blue: DAPI nucleus staining.



B

B: MTT assay demonstrating cell survival and proliferation of ADSCs on DP and PLGA.



Confocal microscopy. 3D images of FDSCs growth on PLGA and DP demonstrating a superior cell growth and penetration on PLGA.

The Effect of porosity on tissue ingrowth and vascularization in electrospun hybrid scaffolds for bladder regeneration

M. Horst , V. Milleret , S. Noetzli , R. Gobet , T. Sulser , D. Eberli

Scaffold porosity governs cellular infiltration, tissue ingrowth and early revascularization which promotes oxygenation of the graft. We investigated the role of porosity of hybrid scaffolds consisting of bladder acellular matrix (BAM) and electrospun poly(lactide-co-glycolide) (PLGA) mimicking morphological characteristics of the bladder wall. Scaffolds with different porosity seeded with smooth muscle cells (SMCs) were evaluated in a cystoplasty model.

Ex vivo evaluation showed an increased cell proliferation on scaffolds with increased porosity (CSS) as compared to single-spun scaffolds (SSS). *In vivo* histology revealed a bladder wall-like structure with urothelial lining, and SMC infiltration. We were able to demonstrate that increased scaffold porosity significantly enhances cell infiltration and revascularization in bladder TE.

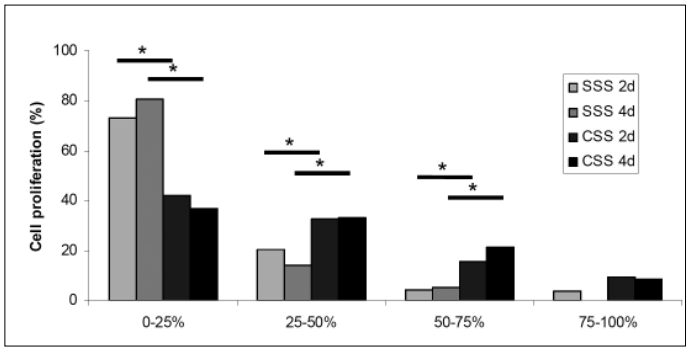


Fig 1: In vitro *seeding: Infiltration depth of SMCs into SSS and CSS showing a deeper infiltration in the more porous scaffolds after 2 and 4 days*

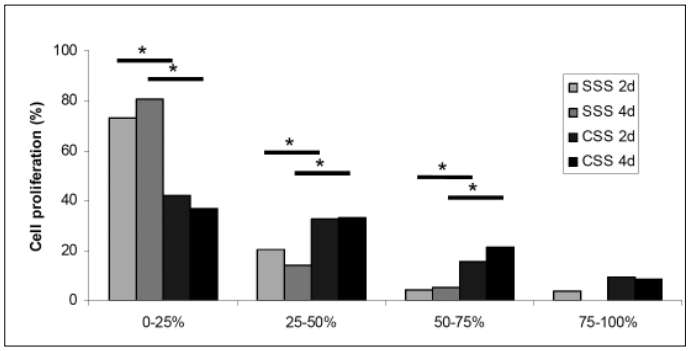


Fig 2: Histological evaluation: Micro vascular density showing significantly increased vascularization of CSS after 2 and 4 weeks.

Muscle Precursor Cells for the treatment of fecal incontinence

L. Brügger, R. Inglin, D. Candinas, D. Eberli

The surgical repair of external anal sphincter is still limited today.

We hypothesize that autologous Muscle Precursor Cells (MPC) injected into the damaged anal external sphincter muscle are able to form new functional muscle tissue. We demonstrated that rodent MPCs were reproducibly harvested, cultured and expanded *in vitro*. MPC-injection for anal sphincter reconstruction resulted in improved sphincter contraction in response to electrical stimulation *(Figure 1)*. We conclude that cell therapy using cultured MPCs might be a promising option for the treatment of anal sphincter insufficiency in near future.

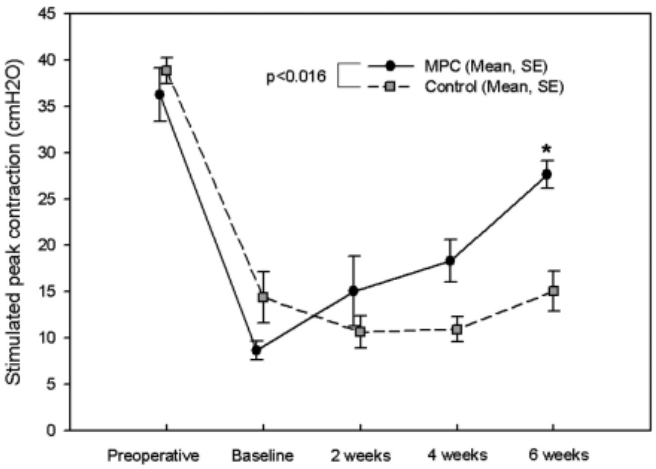


Figure 1: Asterisk in*dicates significant difference from baseline.*

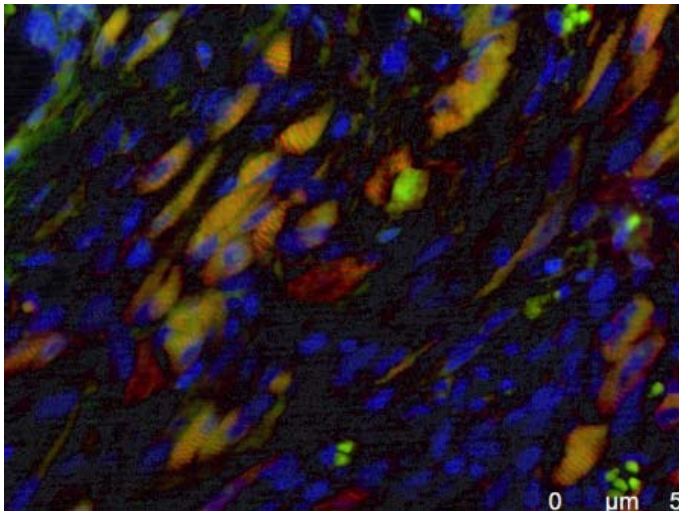


Figure 2:
Newly formed tissue with myofibers demonstrated by immunostaining with anti-α-actinin (red) and tracing of injected MPCs with PKH-67 (green). Nuclear counterstaining with DAPI (blue).

In vivo evaluation of O2 releasing suture material for improved wound healing in hypoxic environments

R. Inglin, B. Harrison, L. Brügger, T. Sulser, D. Candinas, D. Eberli

O2 plays a pivotal role in wound healing. We therefore have developed an O2-releasing suture material and evaluated its influence on healing of hypoxic colon tissue.

A PGA suture material was coated with PLGA containing oxygen-producing calcium peroxide (CPO) nanoparticles. To evaluate the limitations of our approach Lewis rats underwent an ischemia induction of a bowel segment. Oxygen-producing sutures promote anastomotic healing even in challenging environments, and may be clinically used under critical wound conditions in near future.

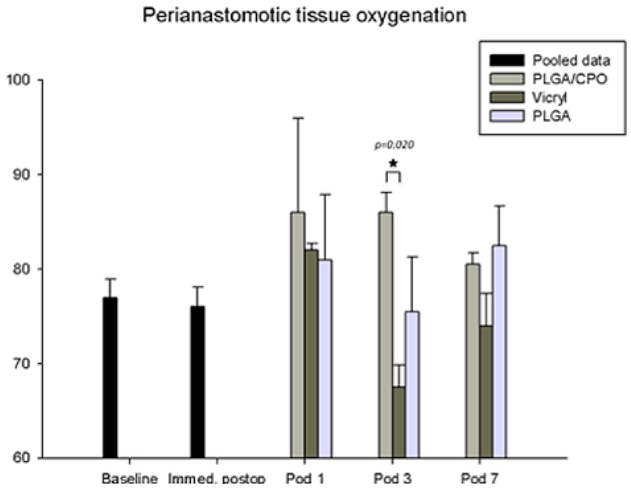


Figure 1 Significantly higher perianastomotic tissue oxygenation in anastomoses performed with O2-producing sutures (PLGA/CPO) compared to untreated Vicryl at postoperative day 3 (p=0.020). Baseline = measurement performed at the presumed site of the anastomosis, before induction of the ischemia. Immed. postop. = measurement performed immediately after confection of the anastomosis in a colon segment with previously induced ischemia as described before.

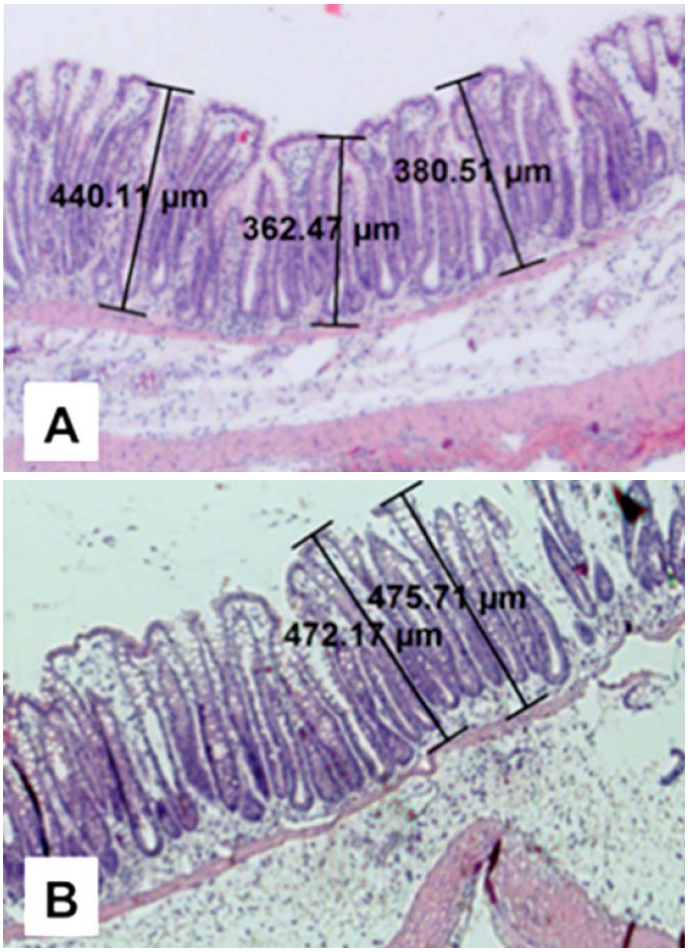
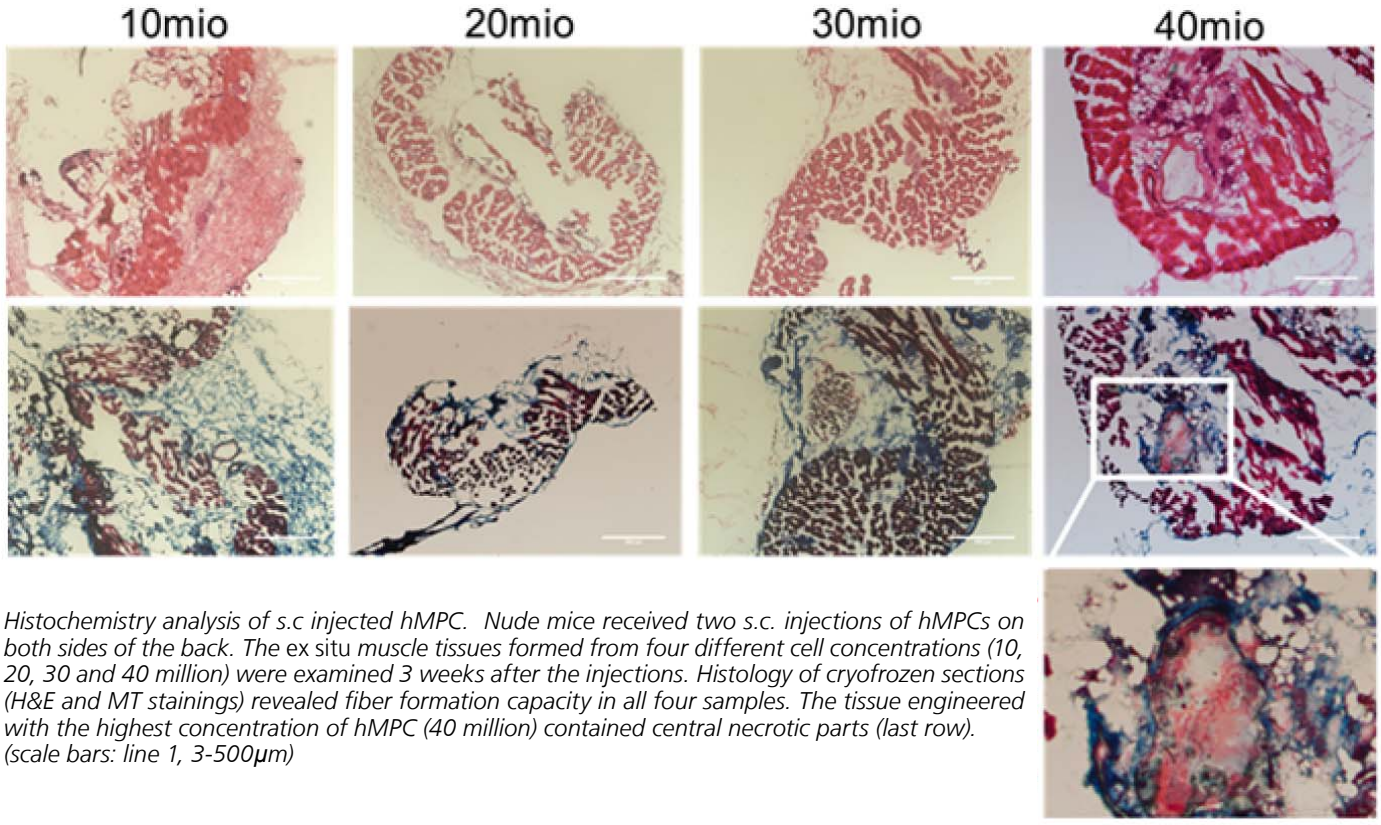


Figure 2 Significantly thicker mucosa and, hence, better wound healing in anastomoses sutured with O2-producing (B) compared to untreated Vicryl suture (A).

Volume limitations in cell therapy for muscle engineering

D. Haralampieva, D. Eberli

One of the key obstacles that need to be considered for engineering of functional muscle tissue is the survival of the injected cells. In this study we therefore first assessed the volume limitations of bioengineered muscle tissue by injecting increasing numbers of cells and investigating the tissue formation and function after harvest. Our study demonstrates that hMPCs are a suitable cell source for engineering muscle tissues. Although myography (organ bath) showed an enhanced functionality with increased cell density, histological analysis revealed that injections with more than 30 million cells will lead to early central tissue necrosis.

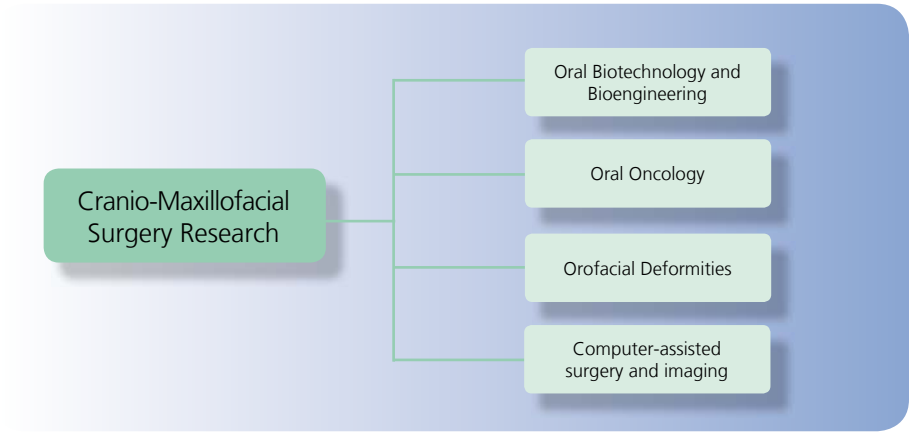


Histochemistry analysis of s.c injected hMPC. Nude mice received two s.c. injections of hMPCs on both sides of the back. The ex situ *muscle tissues formed from four different cell concentrations (10, 20, 30 and 40 million) were examined 3 weeks after the injections. Histology of cryofrozen sections (H&E and MT stainings) revealed fiber formation capacity in all four samples. The tissue engineered with the highest concentration of hMPC (40 million) contained central necrotic parts (last row). (scale bars: line 1, 3-500µm)*

Collaborations:

- Prof. Dr. Benjamin Harrison, PhD, Wake Forest University School of Medicine, Winston-Salem
- Prof. Dr. Rita Gobet, Division of Pediatric Urology, University Children's Hospital Zurich
- PD Dr. Heike Hall-Bozic, Department of Materials, Federal Institute of Technology, Zurich, Switzerland
- Prof. Dr. Grégoire Courtine, Center for Neuroprosthesis, EPFL, Lausanne
- Prof. Dr. Attila Becskei, Institute of Molecular Biology, University of Zurich
- Prof. Dr. Christoph Handschin, Biozentrum, Focal Area Growth and Development, University of Basel
- Dr. Stefano Ferrari, PhD, Institute of Molecular Cancer Research, University of Zurich
- Prof. Dr. Simon Ametamey, Federal Institute of Technology, Zurich, Switzerland
- Prof. Dr. Markus Rudin, Inst. f. Biomedizinische Technik, Universität und ETH Zürich, Schweiz
- Prof. Dr. Janos Vörös, Institut f. Biomedizinische Technik, Federal Institute of Technology, Zurich, Switzerland

2.8 Cranio-Maxillofacial Surgery Research




Prof. Dr.
Franz E. Weber,
PhD


Prof. Dr. Dr. dent.
Klaus W. Grätz,
MD, Director


Prof. Dr. Dr. dent.
Joachim
Obwegeser,
MD

2.8.1 Oral Biotechnology and Bioengineering


PD Dr. Dr. dent.
Astrid Kruse,
MD


PD Dr. Dr. dent.
Heinz-Theo
Lübbers,
MD


Dr. Dr. dent.
Christine
Jacobsen,
MD


Dr. Dr. dent.
Marius Bredell,
MD


Dr.
Chafik Ghayor,
PhD


Dr.
Lindsay Sulzer,
PhD


Rita Correro,
M.Sc.


Patrick Hänseler,
M.Sc.


Nisarat
Roungsawasdi,
DDS


Alexander
Tchouboukov,
Lab. Technician


Yvonne
Bloemhard,
Lab. Technician

Bone, cartilage and tooth regeneration

F. Weber, Ch. Ghayor, L. Karfeld-Sulzer, N. Roungsawasdi, B. Gjoksi, B. Siegenthaler, D. Waldvogel, O. Schätti, A. Tchouboukov, Y. Bloemhard

Growth factor mediated bone regeneration and their enhancers

The main aim of our research efforts is the decrease in the amount of bone morphogenetic proteins (BMP) needed for effect by improving the BMP carriers and using BMP enhancers. In the last years we have identified the small chemical N-methyl pyrrolidone (NMP) which enhances bone regeneration by its enhancing effect on the kinase activity of the BMP/BMP receptor complex for key signalling molecules for bone formation and discovered that the same small chemical reduces osteoclast maturation and osteoclast activity and therefore bone degradation. At present we test the efficiency of NMP for the treatment and prevention of osteoporosis and test scaffolds for the double delivery of BMP and NMP *in vivo*.

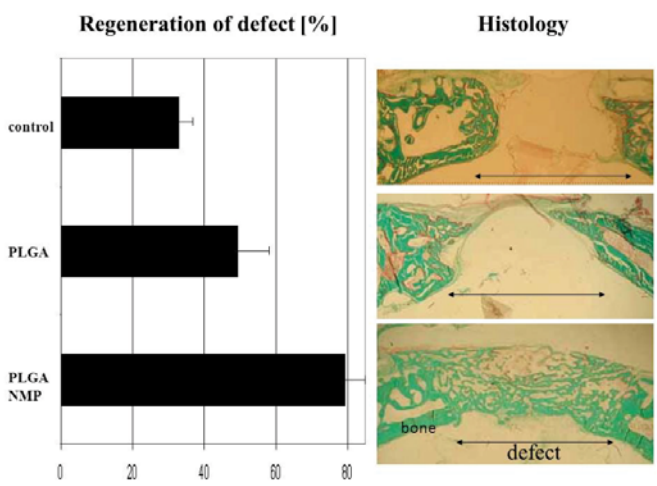


Fig. 1: *Bone regeneration of a calvarial defect in rabbits. Calvarial defects were left untreated (control), treated with a PLGA-based membrane (PLGA), or treated with a PLGA-based membrane releasing NMP. Left: the average regeneration of the defect is shown. Right: the corresponding histology is displayed. The results illustrate that NMP is able to enhance bone regeneration.*

Synthetic hydrogels
Cellular components of bone tissue are osteoclasts, osteoblasts, and osteocytes. To study the interaction between different cell types in bone we have developed a synthetic backbone matrix, where we can incorporate cues in a spatial defined way. In 2011 we generated a bone-like construct composed of osteoblasts, osteocytes and endothelial cells and studied the effect of osteoclasts and osteoblasts on the tube formation of the endothelial cells. Currently we continue to work on the communication between different cell types in bone tissues and a variety of peptides and proteins.

Bone substitute materials
Bone substitute materials are developed to substitute for the use of autologous grafts, which are associated with a second site of surgery, morbidity, pain and additional discomfort for the patient. Since bone is mainly composed of hydroxyapatite the majority of synthetic bone substitute materials contain 60-80% hydroxyapatite. In our group we characterize and develop novel bone substitute materials including titanium based 3D scaffolds. *(Figure 2)*

Biomimetic nano-fiber-based nucleus pulposus regeneration for the treatment of degenerative disc disease
The main objective of this EU-project is to develop a biomimetic substitute used for disc regeneration. To that end, electro-spinning technology is exploited to develop a nano-fiber based, biocompatible, biodegradable, synthetic scaffold mimicking the mechanical properties of the native Nucleus Pulposus for immediate and short term treatment. The synthetic scaffold will be integrated with a bioactive-nano-polymer highly potent in supporting Nucleus Pulposus cells (EPCs) for long-term cure. In addition growth factors will be integrated into the material in a way so that their release suits the needs of this avascular site. Based on our experience with BMPs we have engineered and expressed BMP heterodimers to achieve appropriate binding and release characteristics. *(Figure 3)*

Mechanobiology of cartilage and cartilage tissue engineering
Degenerative joint disease (DJD) of the temporomandibular joint (TMJ) afflicts up to 10% of people with temporomandibular disorders (TMD). The objective of this project is a more specific analysis of the effect that different rolling/plowing conditions have on chondrocyte metabolism. The unique expertise of our partners at the ZZM (Prof. Luigi Gallo) to calculate stress-field areas and to monitor contraction patterns of masticatory muscles, e.g. how long and how often TMJs are loaded, allows us to program a machine which can stress the cartilage explants in a more realistic way than it is normally done in similar experiments. *(Figure 4)*

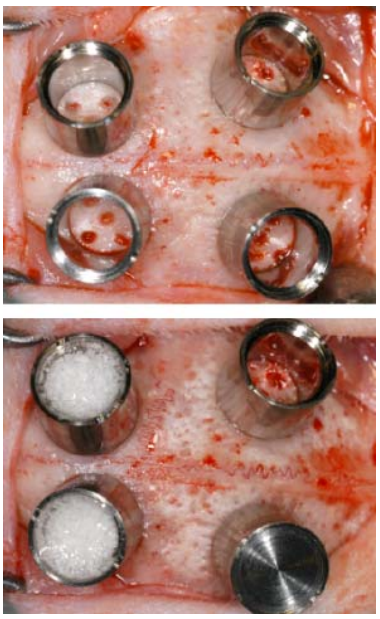


Figure 2: B*one augmentation model using titanium cylinders with lid to study the potential of bone substitute materials in rabbits.*

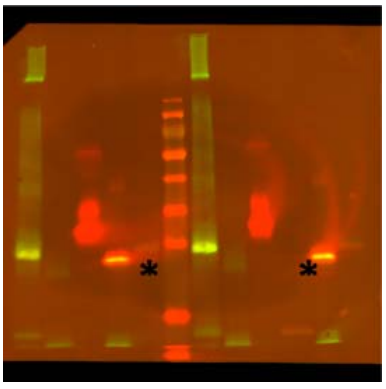


Figure 3: H*eterodimers of BMP-2/7 visualized by the overlay of green and red fluorescent antibodies specific for a single BMP let the band appear yellow (*).*

Pulp regeneration
Tissue regeneration strategies have gained substantial attention in the dental literature over the recent years. Among the regenerative dental procedures, revascularization of a necrotic pulp space appears to be the treatment option that holds the most promise for the immediate future. The procedure is deemed to be especially helpful in children that have teeth with incomplete root formation and that have lost pulp vitality due to caries or trauma. The goal of this project is to establish a new soft tissue in the pulp space that

is capable of continuing hard tissue formation, thus rendering the tooth less prone to fracture and consequent loss. This project is based on our vast experience in hydrogels and growth factor delivery and on a close collaboration to clinical partners at the ZZM (PD Dr. Matthias Zehnder and Prof. Thomas Attin). *(Figure 5)*

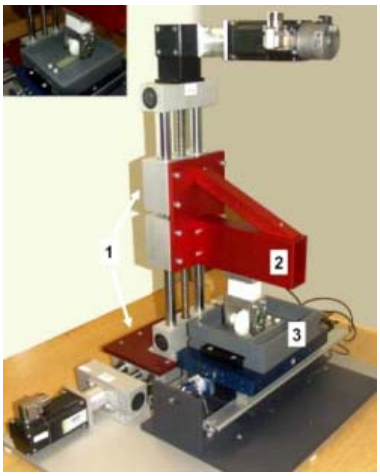


Figure 4: Rolling plowing cartilage test system

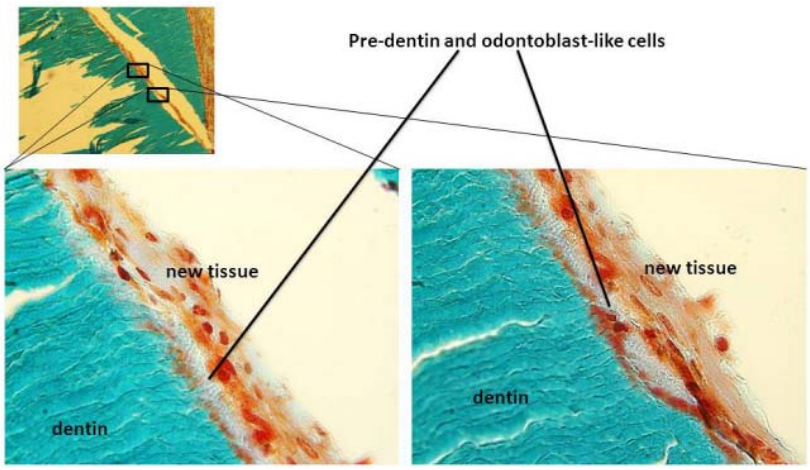


Figure 5: Ingrowth of new tissue into human teeth. A thin layer resembling pre-dentin between mineralized dentin and a cell layer which could contain odontoblast-like cells.

2.8.2 Oral Oncology

M. Bredell, A. Kruse

KFSP Project on Tumor Oxygenation

For many years it has been known that hypoxic tumors have a significantly worse prognosis due to poorer loco regional control and a propensity to disseminate early. Individualizing patients according to their risk base is the aim of any oncological center. This is a large translational research project with developmental (PD.Dr. Martin Wolf), pre-clinical (Prof. Martin Pruschy) and clinical components with the main goal to determine the oxygenation of a tumor in an non-invasive way. Such results should enable us to predict the biological behaviour of a tumor and develop treatment strategies that will cope with hypoxic tumor scenarios at best.

Implant survival in radiated and non-radiated head and neck patients

Implant is a critical part of the masticatory rehabilitation of patients after ablative head and neck surgery as well as radiotherapy. The survival rates of implants in these patients are often less than 70%, compared to over 95% found in non-radiated patients. The primary goal of this study is the development and adaption of a low risk protocol to ensure long term success of implants with stable and functional results. Current endeavors also integrate computer based planning in the treatment protocol to ensure optimal implant placement.

2.8.3 Oral Deformities

J. Obwegeser, Ch. Jacobsen

Until this day, several standardized therapy methods for the surgical correction of craniofacial deformities have been developed. In some severe cases, the method of choice to achieve the best possible functional and aesthetic results is the surgical distraction of syndromic craniofacial deformities. During the last decade, different devices for surgical distraction in the facial area have been developed. But for all of them it is difficult to determine the correct vectors. Additionally some devices show early loosening and unaesthetic scarring, especially in small children. For this a new external distraction device for distraction in Le Fort III level was developed in collaboration with KLS Martin Group. This device was successfully applied in a child with syndromic craniosynostosis.

2.8.4 Computer-assisted surgery and imaging

T. Lübbers

Three-dimensional imaging of the facial surface

Not only in cranio-maxillofacial and plastic surgery, but also in orthodontics and prosthetic dentistry, anthropometry is especially challenging because of the complex structure of faces, which do not allow an accurate assessment with simple measurements. The project is meant to evaluate the precision of 3D facial imaging under clinical circumstances, to integrate this technique into daily clinical routine and to develop evaluation strategies for the acquired data. Simultaneously the introduction of an even superior system designed by ETH / Disney Labs Zurich into clinical setting is an on-going project.

Computer Assisted Surgery (CAS) in oral and cranio-maxillofacial surgery.

The complex three-dimensional geometry and the requirement for a precise facial symmetry are the main challenges in reconstructive maxillofacial surgery. The project therefore focuses not only on the technical parts of Computer Assisted Surgery as e.g. preoperative planning techniques and precision in surgical navigation with its key element of patient registration, but also on clinical guidelines for specific indications and postoperative evaluation of the achieved results.

Three-dimensional radiographic imaging in dental medicine

For the underlying bony structures, multi detector computer tomography as a three-dimensional imaging technique was the classical pendant. The development of cone beam computed tomography brought three-dimensional imaging into the daily business of dental medicine. Only a few years after the introduction of this technique, it has become widely utilized not only in specialized centers but also in general dentistry. Most of the dentist appreciate the new insight into their patient's anatomy and strongly believe into the benefit of this technology. However, despite the broad application of this technology the evidence for most indications is quite unclear. Therefore we started this project to gain evidence for certain applications in surgical and non-surgical indications.

Virtual patient

Next to the above mentioned two imaging techniques there is more data (e.g. intraoral optical scans of the dentition, skin parameters …) that needs to be taken into account and ideally should be integrated into a virtual model of a specific individual. Only based on such an integrative model prediction planning can reach the goal of realistic result forecasts.

Collaborations:

- Department of Fixed and Removable Prothodontics and Dental Material Science, University of Zurich, Switzerland (Prof. Dr. Ch. Hämmerle, PD Dr. Ronald Jung, PD Dr. Daniel Thoma).
- Disney Research, Zürich (Prof. Dr. M. Gross)
- Division of Preventive Dentistry, Periodontology, and Cariology, University of Zurich, Center of Dental Medicine, Zurich, Switzerland (Prof. Dr. T. Attin, PD Dr. M. Zehnder, Prof. Dr. P. Schmidlin).
- Department of Masticatory Disorders, University of Zurich, Switzerland (Prof. Dr. Luigi Gallo)
- Division of Obstetrics (Prof. Dr. Roland Zimmermann, Dr. Martin Ehrbar)
- EPFL Institute of Bioengineering (Prof. Dr. Matthias Lütolf)
- ETH Zürich, Department of Chemistry and Applied Biosciences (Prof. Dr. Wendelin Stark)
- ETH Zürich, Department of Computer Science, Institute for Visual Computing, Computer Graphics Laboratory (Prof. Dr. M. Gross)
- University of Hongkong, Prof. Dr. Roger Zwahlen.
- AO Research Institute, Davos, Switzerland (Prof. Dr. M. Alini).
- Surgical Planning Laboratory, Brigham & Women's Hospital, Harvard Medical School, Boston (MA), USA
- VU University Medical Center, Amsterdam, Netherlands (Dr. M. Helder, Prof. Dr. Th. Smit)
- University of Sheffield, UK (Prof. Dr. Ch. Sammon)

2.9 Surgical Intensive Care Medicine

2.9.1 Neuro Trauma


Prof. Dr.
John F. Stover,
MD


PD Dr.
Markus Béchir,
MD


PD Dr.
Reto Schüpbach,
MD, M.Sc.


Dr.
Miriam Ender,
PhD


Dr.
Jerzy Madon,
MD


Dr.
Stephanie
Klinzing,
MD


Dr.
Renato Lenherr,
MD


Mario Fasshauer


Angela Fendel,
Lab. Technician


Jutta Sommerfeld

Arterial lactate above 2 mM is associated with increased brain lactate and decreased brain glucose in patients with severe traumatic brain injury

R. Meierhans, G. Brandi, M. Fasshauer, J. Sommerfeld, R.A. Schuepbach, M. Béchir, J. F. Stover

Lactate fuels cerebral energy-consuming processes and it is neuroprotective in patients with severe traumatic brain injury (TBI).

In a retrospective study the impact of arterial lactate on brain metabolism was determined. Cerebral microdialysis (glucose, lactate) and blood gas data collected in 20 patients during pharmacologic coma were grouped within predefined arterial lactate clusters (<1, 1-2, >2 mM).

Elevated arterial lactate ≥2 mM was associated with significantly increased brain lactate which coincided with markedly decreased brain glucose despite significantly increased arterial glucose levels. At elevated arterial lactate levels signs of significantly increased cerebral lactate uptake coincided with markedly decreased cerebral glucose uptake. Infused lactate above 50 mM per 24 hours was associated with significantly decreased cerebral glucose.

Increased arterial lactate levels were associated with increased cerebral lactate uptake and elevated brain lactate. At the same time brain glucose uptake and brain glucose were significantly reduced. It remains unclear whether arterial lactate is the driving force for the increased cerebral lactate levels or if the reduced glucose uptake also contributed to the increased cerebral lactate levels. Further studies are required to assess the impact of lactate infusion under clinical conditions.

Changes in plasma phenylalanine, isoleucine, leucine, and valine are associated with significant changes in intracranial pressure and jugular venous oxygen saturation in patients with severe traumatic brain injury

R. N. Vuille-Dit-Bille, R. Ha-Huy, J. F. Stover

Changes in plasma aromatic amino acids (AAA = phenylalanine, tryptophan, tyrosine) and branched chain amino acids (BCAA = isoleucine, leucine, valine) levels possibly influencing intracranial pressure (ICP) and cerebral oxygen consumption (SjvO(2)) were investigated in 19 sedated patients up to 14 days following severe traumatic brain injury (TBI). Compared to 44 healthy volunteers, jugular venous plasma BCAA were significantly decreased by 35% ($p < 0.001$) while AAA were markedly increased in TBI patients by 19% ($p < 0.001$). The BCAA to AAA ratio was significantly decreased by 55% ($p < 0.001$) which persisted during the entire study period. Elevated plasma phenylalanine was associated with decreased ICP and increased SjvO(2), while higher plasma isoleucine and leucine levels were associated with increased ICP and higher plasma leucine and valine were linked to decreased SjvO(2). The amount of enterally administered amino acids was associated with significantly increased plasma levels with the exception of phenylalanine. Contrary to the initial assumption that elevated AAA and decreased BCAA levels are detrimental, increased plasma phenylalanine levels were associated with

beneficial signs in terms of decreased ICP and reduced cerebral oxygen consumption reflected by increased SjvO(2); concomitantly, elevated plasma isoleucine and leucine levels were associated with increased ICP while leucine and valine were associated with decreased SjvO(2) following severe TBI, respectively. The impact of enteral nutrition on this observed pattern must be examined prospectively to determine if higher amounts of phenylalanine should be administered to promote beneficial effects on brain metabolism and if normalization of plasma BCAA levels is without cerebral side effects.

2.9.2 Transplantation

ICG-Liver test versus new biomarkers as prognostic markers for prolonged length of stay in critically ill patients – a prospective study of accuracy in prediction of length of stay in the ICU

U. Wenger, B. Zoller, K. Spanaus, R. Gerster, M. Fasshauer, R.A. Schuepbach, A. Vergopoulos, A. von Eckardstein, M. Béchir

Prognostic abilities of medical parameters, and biomarkers are important for stratifying critically ill patients. Indocyanine green (ICG) plasma disappearance (PDR) is a wide spread easy to use clinical tool for the assessment of liver perfusion and function. Copeptin, MR-proANP and proADM are biomarkers, of which the prognostic value in a heterogeneous ICU population still is unclear. Therefore, we performed this prospective study to compare ICG-PDR measurement with SAPS II, copeptin, MR-proANP, pro-ADM in terms of prolonged length of stay (pLOS) in the ICU.

With the assumptions of a mortality of 7%, a pLOS of 30% and a dropout rate of 10% 110 consecutive patients admitted to the ICU were included in this prospective trial. After receiving written informed consent ICG PDR was measured and the blood sample for the biomarker analyzes were drawn within the first two hours of admission. SAPS II was calculated after the first 24 hours. Primary endpoint was prolonged length of stay (pLOS) in the ICU, defined as more than 3 days of stay in the intensive care unit.

ROC analysis showed an AUC of 0.73 for ICG-PDR, 0.70 for SAPS II, 0.65 for MR-proANP and 0.64 for pro-ADM for prolonged length of stay in the ICU.

This study confirmed ICG measurement to be the best prognostic value in prediction length of stay in critically ill patients whereas novel biomarkers such as copeptin, MR-pro ANP and pro ADM remain to be defined.

Extracorporeal membrane oxygenation: Beneficial strategy for lung transplant recipients

S.R. Cottini, U. Wenger, S. Sailer, M. Wilhelm, R.A. Schuepbach, M. Béchir

Whereas the role of extracorporeal membrane oxygenation (ECMO) became an established therapeutic strategy, its role lung transplant patients, especially the feasibility and safety remains to be clarified.

Data of 15 lung transplant recipients requiring ECMO support was retrospectively analyzed. These required 19 applications of ECMO because or primary graft dysfunction (10 patients), "bridge to transplantation" (5), pulmonary hypertension (3), and severe ARDS (1).

At 28 days, the overall survival was 93% (14 of 15 patients) and 12 of these patients (80%) survived at least 6 months. Complications included acute renal insufficiency with temporary need of renal replacement therapy (53%), bleeding (33%), critical illness polyneuropathy (66%) and reversible thrombocytopenia (73%).

In our small retrospective lung transplant recipients cohort, ECMO was a safe therapeutic approach.

Influence on quality, outcome and ICU osts for lung transplantation after implementation of the new Swiss transplantation law

S. Klinzing, G. Brandi, D.A. Raptis, U. Wenger, U. Weber, P.A. Stehberger, S.R. Cottini, R.A. Schuepbach, M. Béchir

Since July 1st 2007 the Swiss organ allocation system for Donor-lungs has been implemented. The impact of this implementation on patient selection, intensive care unit course, outcome, and intensive care costs is unknown.

The first 42 consecutive lung transplant recipients following implementation of the new act were compared with the previous 37 lung transplant recipients.

Following implementation of the new law, baseline charac-

teristics and cumulative one-year patient survival was comparable in both groups (88.1% vs. 83.8%, p = 0.58). The additional costs per single case increased 35 000 Euros after adoption of the new law.

Conclusion: The new transplantation law is feasible for allocation of lung transplantation. This is the first study to show an increase in ICU costs after the implementation of the Swiss allocation system.

Calculation of ICU costs

	Pre (n=42) Euro	Post (n=37), (%)	p-value
ICU (days) Euros	(441) 882000	(810) 1620000	0.03
ECMO (days) Euros	(5) 5000	(85) 85000	0.04
Ventilation (days) Euros	(263) 132000	(691) 345500	0.88
CRRT (days) Euros	(96) 38000	(437) 174800	0.51
Σ ICU costs Euros	1 057 000	2 225 000	
Σ ICU costs per case Euros	25 000	60 000	
Difference per case Euros		**35 000 Euros**	

Association of intraoperative transfusion of blood products with mortality in lung transplant recipients

D. Weber, S.R. Cottini, P. Locher, U. Wenger, P.A. Stehberger, M. Fasshauer, R.A. Schuepbach, M. Béchir

The impact of intraoperative transfusion on postoperative mortality in lung transplant recipients is still elusive. Univariate and multivariate analysis were performed to investigate the influence of red blood cells (RBC) and fresh frozen plasma (FFP) on mortality in 134 consecutive lung transplants from September 2003 until December 2008.

Intraoperative transfusion of RBC and FFP was associated with a significant increase in mortality with odds ratios of 1.10 (1.03-1.16, p= 0.02) and 1.09 (1.02-1.15, p=0.03), respectively. For more than 4 intraoperatively transfused RBCs multivariate analysis showed a hazard ratio for mortality of 3.8 (1.40-10.31, p=0.003). Furthermore, non-survivors showed a significant increase in renal replacement therapy (36.6% vs. 6.9%, p<0.0001), primary graft dysfunction (39.3% vs. 5.9%, p<0.0001), postoperative need of extracorporeal membrane oxygenation (26.9% vs. 3.1%, p=0.0019), sepsis (24.2% vs. 4.0%, p=0.0004), multiple organ dysfunction syndrome (26.9% vs. 3.1%, p<0.0001), infections (18.1% vs. 0.9%, p=0.0004), retransplantation (12.1% vs. 6.9%, p=0.039) and readmission to the ICU (33.3% vs. 12.8%, p=0.024).

Intraoperative transfusion is associated with a strong negative influence on outcome in lung transplant recipients.

Pretransplant dyslipidaemia determines outcome in lung transplant recipients

U. Wenger, S.R. Cottini, G. Noll, St. Arndt, R.A. Schuepbach, M. Béchir

In the general population, dys- and hyperlipidemia are associated with an increased risk for cardiovascular diseases, as many studies (e.g. publications from the Framingham Heart Study) have shown. Less is known about the role of lipids in the field of transplantation, especially in lung transplant recipients. A systematic chart review on 172 consecutive adult lung recipients transplanted from January 2000 to December 2008 was performed. The total mortality in the observational period was 25% (36 patients overall). In univariate analysis mortality was associated with increased TC/HDL ratio. The non-survivors had on average a 23% higher baseline TC/HDL ratio (3.6 vs. 2.8, HR 2.8, CI 1.2 – 3.5, p = 0.001). There was no association between mortality and TC (p = 0.34), triglycerides (p = 0.34), HDL (p = 0.78) and creatinine (p = 0.73). In a multivariate model the hazard ratio was 1.5 (1.2 – 1.9, p = 0.001) per increase of 0.4 TC/HDL ratio. Therefore the use of statins before transplantation might be beneficial in lung transplant programs.

2.9.3 Coagulation and Inflammation

M. Ender, J. Madon, R.A. Schuepbach

The research efforts of our group aim to understand in more detail the specific pro- and anti-inflammatory pathways in order to identify targets for treating inflammatory driven diseases in clinics. We recently discovered that the protease activated receptor 1 (PAR1), a major extracellular receptor sensing for clotting protease activity harbors two distinct cleavage sites for receptor activation. Cleavage at arginine 41 causes the receptor to mediate proinflammatory stimuli whereas our newly discovered cleavage site at arginine 46 activates PAR1 towards protective, antiapoptotic and vascular barrier protecting pathways *(Figure 1).* This novel finding opens up therapeutic options on how PAR1 could be pharmacologically used in inflammatory driven diseases such as sepsis

Supported by the Swiss National Science Foundation we synthetized a chimeric clotting protease that efficiently binds to a protein co-localized to PAR1 and that activates PAR1 at the desired arginine 46 cleavage site *(Scheme 1).* Studies on whether such a chimeric protease potentially carries a therapeutic potential are currently under investigation.
In a related but complementary effort we are currently testing whether desired antiinflammatory effects could also be achieved by activating PAR1 through antibody fragment tethered PAR1 activating peptides

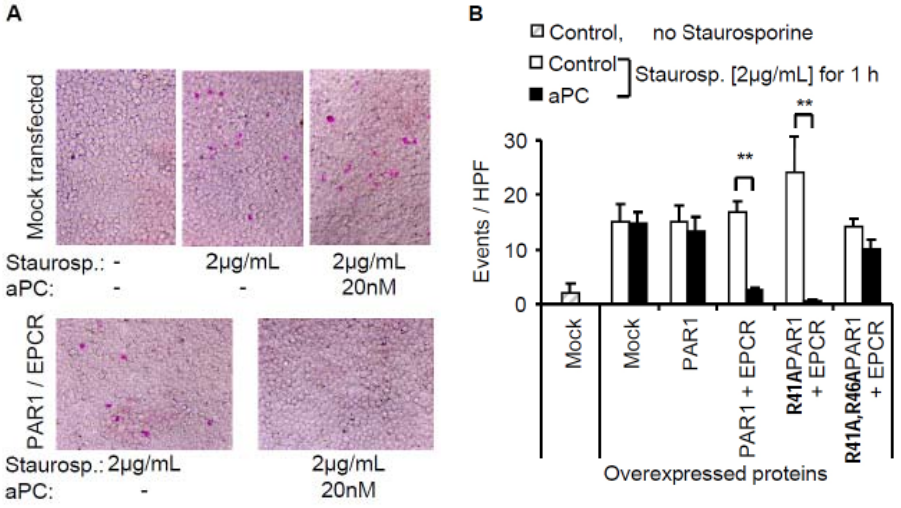
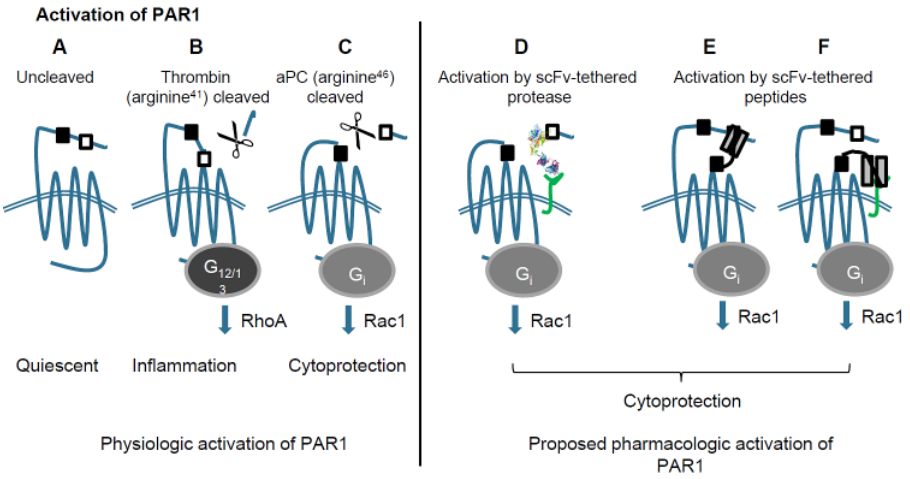


Figure 1: *Biological effects of R46 cleaved PAR1 (A) 293T cells transfected with the indicated expression plasmids ≥48h before experiments were incubated with agonists and staurosporine as indicated and Bengal Rose stained cells were visualized. (B) Transiently overexpressing 293T cells were incubated with hirudin alone (open bar) or aPC (20nM; closed bar) for 16h before quantification of staurosporine induced toxicity. Experiments were repeated at least 3 times, typical sets shown, N=4, **P<0.01. (Data has been published, Schuepbach R.A. et al., JTH 2012)*

Scheme 1: *Activation of PAR1: Physiologically PAR1 is activated by removal of a short N-terminal peptide. The novel N-terminus serves as a tethered activation ligand. (B) Whereas thrombin cleaves at arginine 41 and uncovers a inflammation inducing ligand (open box) activated protein C (aPC) (C) cleaves at arginine 46 and provides a cytoprotective ligand (black box). (D-F) Proposed mechanisms for pharmacological activation of PAR1. (D) Activation by scFv tethered aPC. High (µM concentrations of soluble peptides homologous to the physiological tethered PAR1 ligands have been shown to induce signaling (not shown) We propose to tether ligand peptides to scFv (single chain antibodies) targeting (E) either PAR1 itself or (F) a cell surface protein colocalized to PAR1 (endothelial protein C receptor; EPCR; green).*



We found that not only human but also pathogen derived proteases cleave PAR1. Group A Streptococcus secretes the potent cysteine protease SpeB that cleaves PAR1 at lysine 43. As a result, procoagulant effects of thrombin on platelets is among other effects silenced *(Figure 2).* Further details are currently being investigated.

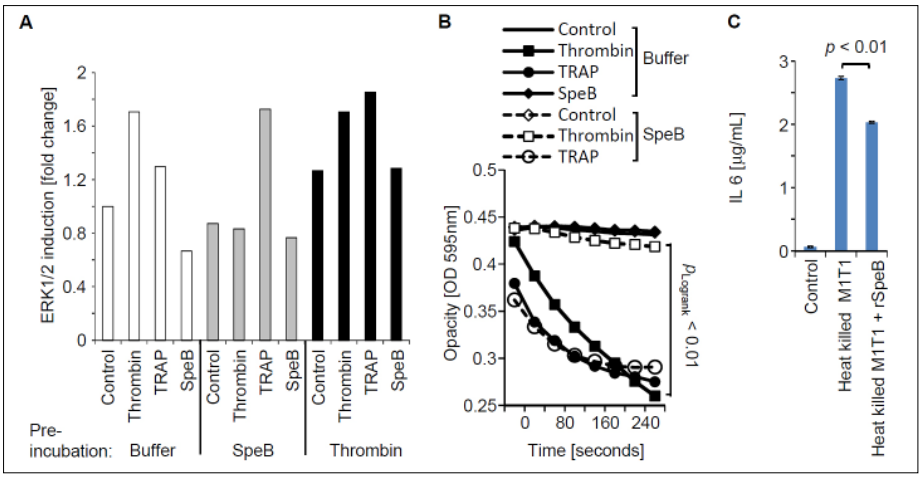


Figure 2: SpeB interferes with PAR1 induced cellular responses: (A) EA.hy926 endothelial cells were incubated with given agonists after a pre-incubation with buffer alone, recombinant SpeB or thrombin before given agonists were added. Phosphorylation of ERK1/2 was analyzed by quantitative Western blotting. (B) Turbimetric quantification of platelet aggregation (lower OD means more aggregates). Washed platelets were incubated with either buffer alone (closed symbols) or recombinant SpeB (open symbols) before given agonists were added and aggregation was quantified. C) EA.hy926 endothelial cells were incubated without or with heat killed non SpeB expressing M1T1 to which either no or rSpeB was added. IL 6 was quantified in the cell's supernatant.

Through collaboration we contributed models and knowledge allowing to study inflammatory driven vascular leakage in viral infection *(c.f. H. Frebel et al. J.Exp.Med. 2012;)* and systemic inflammation caused by bacteria *(c.f. A. S. Zinkernagel et al. J.Innate.Imm. 2012; S. Uchyama et al. PLoS Pathog 2012).*

Collaborations:

- PD Dr. A. Zinkernagel; Klinik für Infektionskrankheiten und Spitalhygiene, UniversitätsSpital Zurich, Switzerland.
- Prof. Dr. A. Oxenius, Institute of Microbiology, ETH Zurich, Zurich, Switzerland.
- Prof. Dr. M. Riewald, Department of Immunology and Microbial Science, The Scripps Research Institute, La Jolla, CA, USA.

2.10 Animal Welfare in biomedical Research

PD Dr.
Margarete Arras,
DVM

Dr.
Nikola Cesarovic,
DVM

Flora Nicholls,
Dipl. biol.

Andreas Rettich,
Dipl. biol.

Paulin Jirkof,
Dipl. nat.

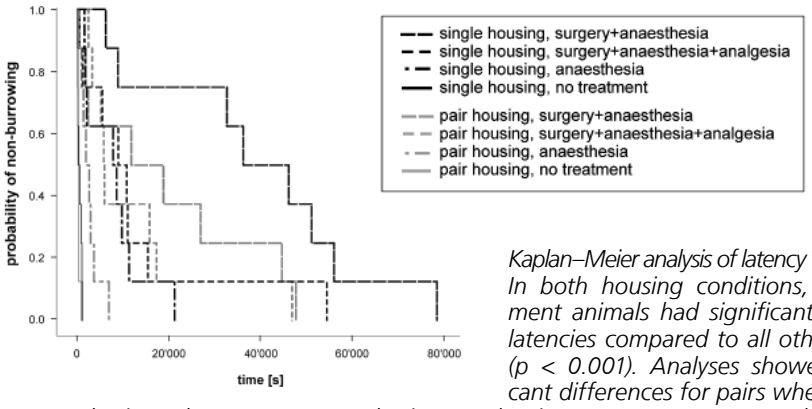
Dr.
Thea Fleischmann,
DVM

The prevention and treatment of pain and distress in laboratory animals is a major concern of animal welfare. In our studies we develop physiological and behavioural parameters to assess pain, distress and general condition in laboratory mice. These tools allow us to optimize anaesthesia and pain relief, provide effective postoperative care and the optimal housing conditions after invasive procedures in mice.

Postsurgical pain and recovery in laboratory mice

P. Jirkof, N. Cesarovic, T. Fleischmann, F. Nicholls, A. Rettich, M. Arras

Short-term individual housing is a standard procedure after surgery but may increase vulnerability to surgical stress or interfere with postsurgical recovery. Better performance in the burrowing test indicates that animals cope better with surgical stress when housed in groups and/or in their familiar environment.

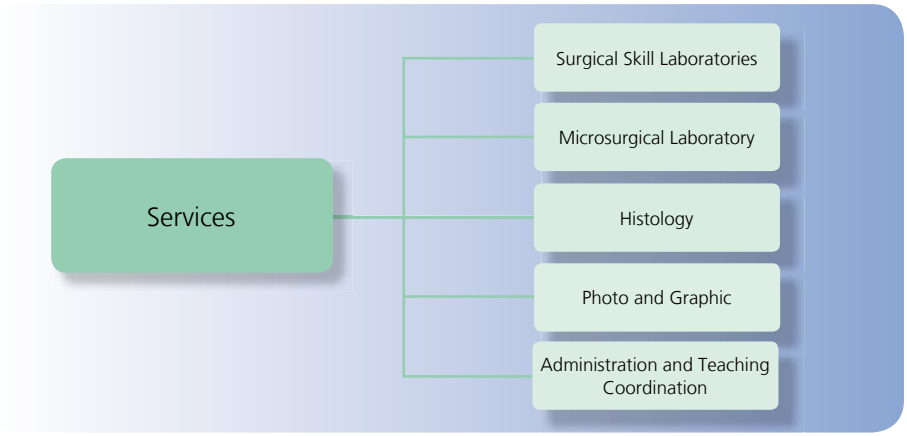


Kaplan–Meier analysis of latency to burrow. In both housing conditions, no-treatment animals had significantly shorter latencies compared to all other groups ($p < 0.001$). Analyses showed significant differences for pairs when surgery + anaesthesia and surgery + anaesthesia + analgesia groups were compared to anaesthesia group ($p = 0.03$; $p = 0.014$), but a significant difference only in single-housed mice when comparing surgery + anaesthesia and anaesthesia groups ($p = 0.003$). Latencies were mainly shorter for pairs compared to single-housed mice, which was significant after surgery + anaesthesia and anaesthesia ($p = 0.021$; $p < 0.001$).

Collaborations:

- Department of Cardio-Vascular Surgery, University Hospital Zurich, PD Dr. M. Emmert, Dr. M. Gessat, Dr. S. Sündermann
- Institute of Laboratory Animal Sciences, University of Zurich, A. Rettich
- Experimentelle Chirurgie, BioMed Zentrum, Universitätsklinikum Freiburg, Deutschland, Prof. Dr. Jörg Haberstroh
- Department of Pediatrics, Endocrinology, University Children`s Hospital Zurich, Dr. S. Wüest, PD Dr. D. Konrad
- Institute of Veterinary Physiology, University of Zurich, Prof. Dr. J. Vogel
- Department of Gastroenterology and Hepatology, University Hospital Zurich, K. Leucht, PD Dr. M. Hausmann
- Institute of Neuropathology, University Hospital Zurich, T. Läufer, PD Dr. M. Neumann, Prof. Dr. A. Aguzzi
- Musculoskeletal Research Unit (MSRU), Competence Center for Applied Biotechnology and Molecular Medicine (CABMM), Equine Hospital, Vetsuisse Faculty, University Zurich, Prof. Dr. vet. med. Brigitte von Rechenberg, ECVS, Dr. vet. med. Katja Nuss
- Orthopedic Research, Balgrist University Hospital, Zurich, Dr. Knut Husmann

3. Services



3.1 Surgical Skill Laboratories

Surgery requires a number of practical and manual skills that can be trained in skill laboratories. In our facilities which are open to all members of the department we provide a number of tools and machines in a surgical environment. To perform operations under conditions similar to the clinical situation, technical help is provided by our staff which is also responsible for the maintenance of our facilities.

3.2 Microsurgical Laboratory

The microsurgery laboratory is a separate section in which several operatingmicroscopes are available to all members of the department requiring special equipment. Maintenance of this laboratory includes all aspects of preparation of surgical instruments, sterilization and handling of waste materials. In addition, an intravital microscope including video equipment is available. This facility also provides for histological work-up.

3.3 Histology

The laboratory for Histology provides a histological work-up from preserved specimen to sectioning and staining. The laboratory contains an embedding machine, several microtomes and staining devices. Several techniques including paraffin embedded, frozen and plastic embedded tissue can be processed.

3.4 Administration

- Administrative office management
- Financial accounting of the Research Divison
- Organisation, planning and coordination of workshops and vocational training
- Workshop, tutorials and seminars
- Quarterly reports
- Meeting organisation and coordination
- Personnel administration

3.5 Teaching Coordination

- Coordination and organization of the learning and teaching units in the Department of Surgery from 1st to 6th years of study including lectures and clinical courses in the compulsory part of the curriculum as well as in the electives.
- Coordination, organization of the clinical rotations during the 5th year of study.
- The work is done in cooperation with the University of Zurich and the UniversityHospital Zurich for the Department of Surgery.

PD Dr.
Margarete Arras,
DVM

Dr.
Nikola Cesarovic,
DVM

Nico Wick,
Photographer

Lea Schütz,
Photographer

Carol De Simio,
Scientific
Illustrator

Stefan Schwyter,
Scientific
Illustrator

Pia Fuchs,
Lab. Technician

Donata Gröflin,
Teaching
Coordination
Division of
Surgical Research

Corinne Renold,
Teaching
Coordination
Division of
Surgical Research

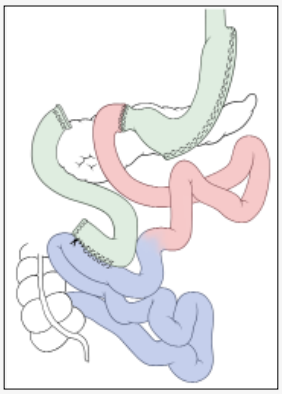
Susanne Frehner,
Administration
Division of
Surgical Research

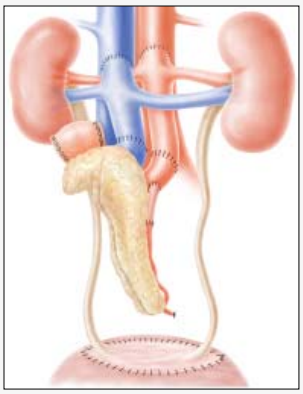
3.6 Photo and Graphic Services

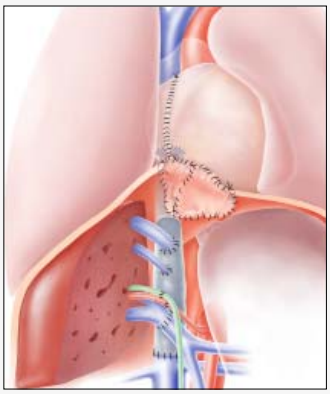
A quick, flexible, versatile and professional service.

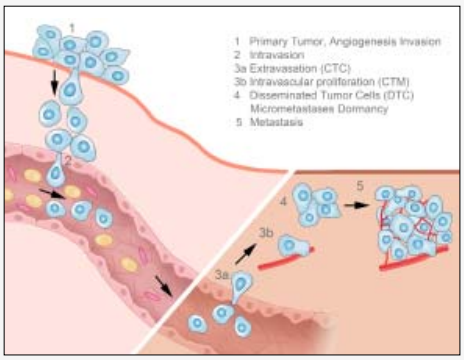
We offer

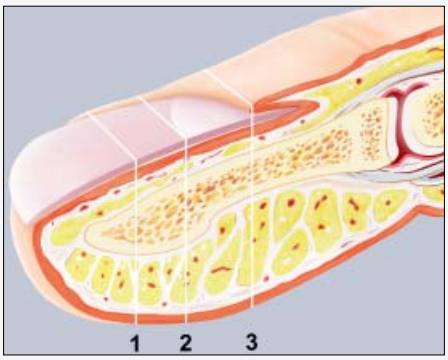
- photographic documentation of patients and events
- technical photography, on location or in our studio
- graphic and design of illustrations for papers and books
- reproductions from any original
- layout of printing matters
- preparation of files for external printing
- print service
- cutting and converting of movie-files for presentation
- construction and maintainance of websites
- maintainance of the digital image archives











4. Events and Workshops at the Division of Surgical Research 2012

8th Swiss Stem Cell Network Meeting, Zurich February 10

11th Day of Clinical Research, April 19

Surgical Suture Skills Course, March 14 / 15

3rd Symposium of the Swiss Clinical Trial Organisation, June 14

Inauguration Animal Hybrid Surgical Room, June 28

Good Bye Martha Bain, October 30

CTC Symposium " Das neue Humanforschungsgesetz", November 1

Aesculap Suture Skills Course, November 11

Christmas Party, December 15

5. Publications 2012

Cardiovascular Surgery

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Plastic, Hand and Reconstructive Surgery

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  Tissue engineered bone grafts based on biomimetic nanocomposite PLGA/amorphous calcium phosphate scaffold and human adipose-derived stem cells.
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6. Grants 2012

Cardiovascular Surgery

Source	Title of Project	Project Leader
Swiss National Science Foundation	Advanced Cell Therapies for Cardiac Repair-SPUM	S. Hoerstrup
Swiss National Science Foundation	Characterization/evaluation & cell fate in vivo analysis of fetal stem cells used for cardiovascular tissue engineering applications	S. Hoerstrup
Swiss National Science Foundation	Prenatal minimally invasive implantation of fetal cell-based autologous living heart valves - a novel approach using naive and amniotic fluid-derived induced pluripotent stem cells	S. Hoerstrup
Commission of the European Communities	Living autologous heart valves for minimally invasive implantable procedures	S. Hoerstrup
Schweizerische Herzstiftung, Bern	Living Heart Valves for the few	S. Hoerstrup
Novartis Stiftung für Biologisch-Medizinische Forschung	miRNA manipulations to improve the regenerative potential of bone-marrow derived MSCs	S. Hoerstrup
SCIEX-NMSch Fellowship	SCIEX Fellowship Jaro Slamecka	S. Hoerstrup
Unitectra Technologietransferfonds	Targeting Fibroblast Activation Protein to prevent Infarction	S. Hoerstrup
Schweiz. Gesellschaft für Kardiologie	Cardiovascular Biology Prize of the Swiss Society for Cardiology	S. Hoerstrup
KTI, Bern	Proof of concept: Human FAP-targeting Antibodies as Infarction Prevention Medications	S. Hoerstrup
EMDO Stiftung, Zürich	Establishment of a novel SCID/g heterotopic working heart transplantation model for in vivo investigation of tissue engineered heart valve remodelling mechanisms and cell fate	S. Hoerstrup
Commission of the European Communities	European Clinical Study for Application of Regenerative Heart Valves	M. Hübler
Swiss National Science Foundation	Biomechanische Simulation der katheterbasierten Aortenklappenimplantation	M. Gessat
Philips Electronics North America Co.	Evaluation and development of image-based technologies for cardiovascular therapy	V. Falk
Terumo Europe NV Leuven/Belgium	Oxygenator performance and biocamptibility comparison of two commercially available coated oxygenators and circuits	M. Wilhelm
Atricure Inc.	Cosgrove-Gillinov Clip Study	S. Salzberg
Schweizerische Herzstiftung, Bern	Mitral Valve Annuloplasty: from diagnostic tools to predictive simulation	S. Jacobs
CardioGard Medical Ltd./Israel	CardioGard: Klinische Studie zur Begutachtung der Sicherheit und der Funktion der CardioGard-Kanüle	Ch. Starck
St Jude Medical Coordination Center BVBA, Zaventem Belgium	Trifecta Durability Study. Studie zur Langlebigkeit von Trifecta	V. Falk
Schweizerische Herzstiftung, Bern	The clinical value of 3D template based planning for percutaneous aortic valve implantation	M. Gessat
Edwards Lifesciences / USA	Carpentier-Edwards / Perimount Magna / Mitral Periocardial Bioprostheses	V. Falk
Valtech Cardio Ltd., Israel	"Valtech Cardinal adjustable Semi-Rigid annuloplasty Ring System for Treatment of Mitral Valve Regurgitation in Open Surgical Repair" and "Valtech V-Chordal adjustable System for chordal repair in Mitral Valve Insufficiency due to leaflet prolaps"	V. Falk
Medtronic Ventor Technologies / NL	Medtronic Engager Feasibility and Pivotal Trial	V. Falk

Visceral & Transplant Surgery

Source	Title of Project	Project Leader
Hepatobiliary Laboratory		
Swiss National Science Foundation	Serotonin and regeneration in the normal, old and diseased liver	P.-A. Clavien
Swiss National Science Foundation	Reversible portal vein embolization for safer liver surgery and transplantation	M. Lesurtel
Swiss National Science Foundation	Uzbekistan International Cooperation: Transition to Modern Hepato-Pancreato-Biliary Surgery	M. Lesurtel
Swiss National Science Foundation	Establishment of a Morbidity Index to Assess Surgery	K. Slankamenac
Swiss National Science Foundation	Adjuvant gemcitabine versus NEOadjuvant gemcitabine/oxaliplatin plus adjuvant gemcitabine in resectable Pancreatic Cancer: a randomized multicenter phase III study (NEOPAC study).	P.-A. Clavien
Swiss National Science Foundation	Konditionierung mit volatilen Anästhetika in der Lebertransplantation.	S. Breitenstein
Swiss National Science Foundation	Machine liver perfusion for protection from biliary injury	P. Dutkowski
Swiss National Science Foundation	Metal Nanomagnets - towards single cell surgery	B. Beck-Schimmer, P.-A. Clavien
National Institutes of Health (R01DK 092608-01A1)	Intestinal satiation in Roux-en-Y gastric bypass rats: brain mechanisms and sex differences	M. Bueter (Co-Principal Investigator)
ZIHP - Zurich Center for Integrative Human Physiology	Immediate improvement of lipid metabolism, liver and endothelial function after Roux-en-Y gastric bypass (RYGB) surgery in obese humans and rodents	M. Bueter (Co-Applicant)
ETH Zürich as the Swiss Leading House for the SSSTC	Sino-Swiss Science and Technology Cooperation	P.-A. Clavien
University of Zurich (Klinische Forschungsschwerpunkte)	Molecular Imaging Network, Zürich	M. Lesurtel / A. Boss
Kurt und Senta Herrmann Stiftung, Vaduz, FL	Targeting Reactive Oxygen Levels	K. Lehmann
Olga Mayenfisch Stiftung	Versagen der Leberregeneration nach ausgedehnter Leberresektion als Ursache des 'Small-for-Size Syndroms'	K. Lehmann
Sassella-Stiftung	Adjuvant gemcitabitabine versus neoadjuvant gemcitabine / oxaliplatin and adjuvant chemotherapy with gemcitabine in patients with resectable pancreatic cancer	P.-A. Clavien
Pancreatitis Laboratory		
Swiss National Science Foundation	Role of serotonin in inflammation, repair and regeneration of the pancreas	R. Graf
Velux Stiftung	The role of macrophages in chronic pancreatic inflammation	R. Graf
Gottfried und Julia Bangerter-Rhyner-Stiftung	Serotonin in Pancreas	R. Graf
Gebert Rüf Stiftung, Basel	PSP and Sepsis	R. Graf
Amélie Waring Stiftung	Pathophysiology of chronic pancreatitis	R. Graf

Trauma Surgery

Source	Title of Project	Project Leader
Bayer (Schweiz) AG	Xamos-Xarelto in the Prophylaxis of post surgical venous thromboembolism after elective major orthopedic surgery of hip or knee	H.-P. Simmen
Theodor und Ida Herzog-Egli-Stiftung	Prevention of heterotopic ossification - new approaches	H.P. Simmen / C. Werner
B. Braun AG	Randomized, double-blind, controlled clinical trial on the antispetic efficacy and tolerability of Lavasept 0.04% on acute traumatic wounds	H.P. Simmen / C. Werner
Dr. h.c. Robert Mathys Stiftung	Prevention of heterotopic ossification - new approaches	C. Werner
Synthes GmbH	Klinische Nachkontrollstudie. Proximaler Humerus, Schraubenosteosynthese medialer Schenkelhalsfrakturen	H.P. Simmen / C. Werner
Emdo Stiftung Zürich	Neue Strategien in der Prävention Heterotoper Ossifikationen	C. Werner
AO Research Fund	Assessment of soft-tissue and periosteal microcirculation in severly open fractures using othogonal polarization spectral imaging	G. Wanner
SUVA Luzern	FG-Zellweger - Knochenmetabolismus	G. Wanner
Stiftung für wissenschaftl. Forschung an der UZH	Genetic profiling of severely injured patients - transcriptomics of inflammation for opening the "window of opportunity"	G. Wanner
CABMM (Center of Applied Biotechnology and Molecular Medicine) UZH	Identification of tenocyte specific markers in the horse	P. Cinelli
Olga Mayenfisch Stiftung	From pluripotency to differentiation: the role of Pramel7 in murine embryonic stem cells	P. Cinelli

Plastic, Hand and Reconstructive Surgery

Source	Title of Project	Project Leader
Swiss National Science Foundation	Characterization of the vascularization of skin grafts, skin substitutes and biomaterials *in vivo* and identification of the vascular mechanisms	N. Lindenblatt
Swiss National Science Foundation	Breast tissue reconstruction: Potential and therapeutic implications of mesenchymal stem cells	J. Plock
Forschung und Nachwuchsförderung der Universität Zürich	Sehnenreparatur mit einem reversibel expandierbaren Schlauch - Kaninchen Achillessehnenmodell *in vivo*	J. Buschmann
Wolfermann-Nägeli-Stiftung	Sehnenreparatur mit einem reversibel expandierbaren Schlauch - Kaninchenmodell *in vivo*	J. Buschmann
Forschung und Nachwuchsförderung der Universität Zürich	Hauttransplantate	N. Lindenblatt
AbMedica, Lainate (Italy)	Sehnenreparatur mit einem reversibel expandierbaren Schlauch - Kaninchenmodell *in vivo*	J. Buschmann
Swiss Life Research Grant, Zurich	Skin grafting and tissue engineering of skin substitutes in burn surgery - what we can learn from nature	N. Lindenblatt
Swiss National Science Foundation	Cellular and molecular mechanisms of vascular maturation for therapeutic angiogenesis	*A. Banfi, Basel; N. Lindenblatt (Co-Applicant)*

Plastic, Hand and Reconstructive Surgery

UZH - Matching Funds	CAM Assay für high-field MRI (DFL 1127)	J. Buschmann
Hartmann-Müller Stiftung	Sehnenreparatur unter Zuhilfenahme eines mit PDGF-BB bestückten Degrapol-Rohrs	J. Buschmann

Thoracic Surgery

Source	Title of Project	Project Leader
Hartmann-Müller Stiftung Zürich - 2012	Improvement of ischemia-reperfusion injury in muring lung transplants by stem cells via inhibition of DPP4 (Ges-Nr: 1591)	W. Jungraithmayr
Swiss National Science Foundation Förderungsprofessuren	Malignant Pleural Mesothelioma - an integral approach for better outcome	I. Schmitt-Opitz
Swiss National Science Foundation	The role of CD26/DPP IV and SDF-1 in pulmonary ischemic injury in a mouse	W. Jungraithmayr
Swiss National Science Foundation	Immune targeted therapy for lung cancer	S. Hillinger
Swiss National Science Foundation	In-Vivo Bioreactor for the reepithelialization of tissue engineered trachea	W. Weder
Swiss National Science Foundation	Micro Computer-Tomographie für in-vivo Bildgebung	W. Jungraithmayr (Co-Applicant) Applicant: PD Dr. A. Boss
Swiss National Science Foundation	Magnetic resonance imaging for the detection of chronic lung allograft rejection in mouse lung transplantation	W. Jungraithmayr (Co-Applicant) Applicant: PD Dr. A. Boss
Lungenliga Zürich	Rekonditionierung durch Magensäure geschädigter Lungen-transplantate Nicht-Herz-schlagenden Spendern	I. Inci
Dr. Arnold U. u. Susanne Huggenberger-Bischoff Stiftung zur Krebsforschung (Krebsstiftung) Co-applicant	*In vivo* study of the efficacy of a dual phosphatidylinositol-3-kinase (PI3K)-/mTOR-inhibitor in the treatment of malig-nant pleural mesothelioma	W. Weder, I. Schmitt-Opitz
Matching Fund UZH 2011	Malignant Pleural Mesothelioma - an integral approach for better outcome	I. Schmitt-Opitz
Matching Fund UZH 2012	Preclinical investigation of the role of hedgehog signaling on mesothelioma recurrence	I. Schmitt-Opitz
Matching Fund UZH 2011	Prognostic Marker for Malignant Pleural Mesothelioma	I. Schmitt-Opitz
Krebsliga Zürich	Prognostic Marker for Malignant Pleural Mesothelioma	I. Schmitt-Opitz
Krebsliga Zürich	Activity based protein profiling in human lung cancer biopsies	W. Weder, S. Hillinger, S. Arni
Novartis Pharma AG Basel	Identification and validation of drug targets and biomarkers for COPD/emphysema and other end-stage lung disease	W. Weder, S. Hillinger
Matching Fund UZH 2012	Improvement of ischemia-reperfusion injury in murine lung transplants by regenerative stem cells via inhibition of CD26/ DPPIV	W. Jungraithmayr
Matching Fund UZH 2011	Tolerance induction via NK cell mediated elimination of donor antigen presenting cells in mouse lung transplants	W. Jungraithmayr

Urology

Source	Title of Project	Project Leader
Max & Hedwig Niedermayer Stiftung	The Role of Autophagy in the Differentiation of Adipose Derived Stem Cells for Functional Smooth Muscle Bioengineering	D. Eberli
Novartis Stiftung für Biologisch-Medizinische Forschung	Improving human muscle engineering by PGC-1alpha overexpression"	D. Eberli
Klinischer Forschungsschwerpunkt "Molecular Imaging Network Zurich", Co-Applicant	In-vivo characterization of differentiating muscle precursor cells applying multi-modal molecular imaging"	D. Eberli
Swiss National Science Foundation 323230_126230/1-Prolongation	Adult Muscle Progenitor Cells for the Treatment of Urinary Incontinence	D. Eberli
Promedica Stiftung, Chur	Improving human muscle engineering by PGC-1 alpha overexpression	D. Eberli
Medical Faculty - UZH	Research grant from the Medical Faculty for the additional financial support of the Tissue Engineering Laboratory	D. Eberli
Research grant from the Strategic Pool of the "Medizinisches Dekanat", Prof. Grätz	Tissue Engineering Projects "Oxygen and VEGF releasing Biomaterials"	D. Eberli
Baugarten Stiftung, Zürich	MPCs for the treatment of urinary incontinence	T. Sulser, D. Eberli
Swiss National Science Foundation CRSII3_136197 / 1	Improving human muscle engineering by PGC-1alpha expression and molecular imaging using positron emission tomography (PET)	D. Eberli

Cranio Maxilla Surgery

Source	Title of Project	Project Leader
Swiss National Science Foundation	The potenetial of N-methylpyrrolidone to prevent osteoporosis and to enhance bone regeneration	F. Weber
CABMM (UZH) Starting grant	DMA for bone regeneration and inhibition of bone resorption	F. Weber
AO-CMF Grant	Hydrogel-titanium composites for mandibular reconstruction	F. Weber
EU-Grant (FP7-NMP-2009-SMALL-3)	Biomimetic nano-fiber-based nucleaus pulposus regeneration for the treatment of degenerative disc disease	F. Weber for UZH, M. Helder from the University of Amsterdam
Swiss National Science Foundation	Catabolic and anabolic reaction of dynamically loaded chondrocytes under biomimetic conditions	F. Weber from USZ, L. Gallo from ZZM
Swiss National Science Foundation	Artificial mesenchymal progenitor cell niches for bone tissue engineering	F. Weber from USZ, M. Lütolf from EPFL

Intensive Care Unit

Source	Title of Project	Project Leader
Swiss National Science Foundation	Cytoprotection through non Anticoagulant Engineered Chimeric Activated Protein C	R. Schüpbach
Forschung und Nachwuchsförderung der Universität Zürich	Role of PAR1 *in Vascular* Barrier Regulation	R. Schüpbach
Hartmann Müller-Stiftung für medizinische Forschung	Role of PAR1 *in Vascular* Barrier Regulation	R. Schüpbach
Fresenius Kabi (Schweiz) AG Stans	Early fluid resuscitation with balanced HES 130/0.4 (6%) in severe burn injury	M. Béchir
Diverse	Gerinnung	R. Schüpbach
Hartmann Müller-Stiftung für medizinische Forschung	Activation of Protease Activated Receptors by Bacterial Proteases	R. Schüpbach
UBS Wealth Management	Pathophysiologische Relevanz aktivierter Thrombocyten und Einfluss von Noradrenalin auf die Funktion isolierter Thrombozyten nach schwerem Schädel Hirn Trauma	Prof. J.F. Stover
Matching Fund UZH 2011	Regulation of the Endothelial Barrier by the Protease Activated Receptor 1	R. Schüpbach

Veterinary Services

Source	Title of Project	Project Leader
ECLAM-ESLAV Foundation, London	Characterization of isoflurane and sevoflurane for anesthesia of mice and optimization of balanced anesthesia regimens comprising surgical tolerance	M. Arras
privater ext. Gönner (via UBS)	Tierschutz in der biomedizinischen Forschung - Verhaltensänderungen als Schmerzindikatoren bei Labormäusen	M. Arras
BVET Bern (Bundesamt für Veterinärwesen)	Etablierung von effizienten Schmerzbehandlungsmethoden für die Labormaus	M. Arras
BVET Bern (Bundesamt für Veterinärwesen)	Beurteilung von post-operativen Schmerzen anhand von Verhaltensparametern bei Labormäusen	M. Arras

7. Awards 2012

- Benedikt Weber
  Swiss Cardiovascular Biology Award 2012
  Swiss Society of Cardiology
  (Prof. T. Lüscher, Prof. H. Rickli), Lausanne, June 2012 (Prenatally engineered autologous amniotic fluid stem cell-based heart valves in the fetal circulation)
- Benedikt Weber, Maximilian Y. Emmert
  Pfizer Research Award 2012
  Zurich, February 2012 – Category: "Herzkreislauf, Urologie und Nephrologie"
  Award Commission Prof. B. Waeber, Prof. T. Gasser, Zürich 2012 (Injectable living marrow stromal cell-based autologous tissue engineered heart valves)
- Dieter Cadosch
  Award of the Swiss Society of Surgery in Clinical Research 2012; Davos, Switzerland.
  Metal sensitivity in total knee arthroplasty.
- Dieter Cadosch
  Award of Distinction to the Ph.D. Thesis
  "Biocorrosion and Aseptic Loosening of Metal Implants: Novel Pathophysiological Mechanisms".
  University of Western Australia, 2012
- Thomas Wiedl
  Preis der Schweizerischen Gesellschaft für Chirurgie
  Beste experimentelle Publikation
  Activity-based proteomics: Identification of ABHD11 and ESD activities as potential biomarkers for human lung adenocarcinoma
- Stephane Collaud
  Preis der Schweizerischen Gesellschaft für Chirurgie
  Beste klinische Publikation
  Significance of a new fluorodeoxyglucose-positive lesion on restaging positron emission tomography/computed tomography after induction therapy for non-small-cell lung cancer
- D. Eberli
  C.E. Alken-Preis für hervorragende wissenschaftliche Leistungen auf dem Gebiet der Urologie.
  "Hybrid-Biomaterial mit definierten Oberflächenmerkmalen und Wachstumsfaktoren unterstützt die Nervenregeneration beim Blasen züchten"
  C.E. Alken-Preisträgertreffen 2012, München.
- S. Madduri, R. Gobet, T. Sulser, D. Eberli
  C.E. Alken Research Prize (KelmHjälmas First Prize Basic Science)
  Collagen/Silk Fibroin based hybrid Biomaterial with sustained release of Nerve Growth Factor for axonal Regeneration in Bladder Tissue Engineering
  23nd Annual Meeting of the European Society for Pediatric Urology, Zurich, 2012
- L. Brügger, R. Inglin, D. Candinas, T. Sulser, D Eberli
  Felix Largiadèr-Preis
  Stem Cells for the Treatment of Anal Incontinence
  Swiss Surgical Society, Annual meeting 2012
- M. Stölting, Ch. Handshin, A. Becskei, T. Sulser, D. Eberli
  Posterprize Stem Cell Research, Annual Meeting AUA
  In vivo Electromagnetic stimulation supports muscle regeneration after stem cell injection by boosting muscular metabolism and stimulating nerve ingrowth
  Annual Meeting of the American Urological Association, Atlanta
- M. Provenzano
  Paper awarded: J Virol. 2012, 86: 8461-8471 Journal Tipsheet: "New Evidence for Polyomavirus BK Role in Prostate Cancer", the American Society for Microbiology.

- Ch. Tschuor
  Best oral presentation ARS *(Association for Research in Surgery)* – 99. Jahreskongress der SGC, Davos, 20.-22. Juni 2012
  Overcoming regenerative limits in hepatic surgery through primary mitogen induced liver hyperplasia
  Ch. Tschuor, B. Humar, J.-H. Jang, A. Schlegel, P. Limani, K. Lehmann, D.A. Raptis, R. Graf, P.-A. Clavien
- N. Lindenblatt
  One of the Top 3 presentations at the European Plastic Surgery Research Council 2012 – 25.08.2012
  Lindenblatt N, Knapik A, Hegland N, Althaus M, Contaldo C, Calcagni M, Giovanoli P. Vessel Development during skin graft revascularisation – implications for tissue engineering
- Nicholls, F, Fleischmann, T, Jirkof, P, Cesarovic, N, Arras, M
  Poster award
  Assessment of postoperative pain by nest complexity scoring in mice.
  Joint symposium BCLAS/ESLAV/ECLAM, Liège, 2012.
- St. Breitenstein
  Chirurgie Preis - Prix de chirurgie
  Schweizerische Gesellschaft für Gastroenterologie & Schweizerische Gesellschaft für Viszeralchirurgie
- M.Gessat
  Third Prize - Best Paper Award Session Jahrestagung der Deutschen Gesellschaft für Computer- und Roboterassistierte Chirurgie (CURAC), Düsseldorf
  M. Gessat, S.H. Sündermann, N. Cesarovic, T. Frauenfelder, P. Biaggi, D. Bettex, V. Falk, S. Jacobs
  Patientenspezifische Annuloplastieringe für die Mitralklappenkonstruktion

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