Division of Surgical Research

Annual Report 2008

Department of Surgery
University Hospital Zurich
Switzerland


Division of Surgical Research
Department of Surgery
University Hospital
Rämistrasse 100
CH - 8091 Zurich

Content

Preface

Dear Colleagues

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

It is my privilege to present the Annual Report 2008 of the Division of Surgical Research at the Department of Surgery, University Hospital Zurich.

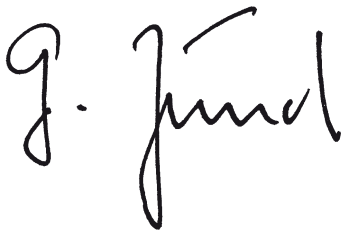
In the year 2008, the two new surgical clinics, (Clinic for Urology and Clinic for Maxillary Surgery) which already joined our division last year, have been fully integrated.
Several personnel changes within the Department of Surgery happened last year. Prof. Otmar Trentz retired in the last summer and Prof. Michele Genoni left the University Hospital. I would like to specially thank Prof. Trentz as head of the department for all the support for the division of surgical research, and I would like to thank Prof. Genoni for his personal support for the cardiovascular research group.

The investments of laboratory equipment made in the past year include the purchase of three Revco ultra low temperature laboratory freezers, one ELISA microplate reader, one mobile video microscope for the operating room, two operating room x-ray tables and one anesthesia unit for the microsurgery room.

For teaching activities, several wet lab events for surgeons and microsurgery classes for surgical residents were offered. The weekly lectures held by the Divisions of Surgical Research at the University Hospital Zurich were regularly attended by the members of our Division and other researchers 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 performance and collaboration.

Yours sincerely



Prof. Dr. med. Gregor Zünd
Head Division of Surgical Research

1. Organisation

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

Prof. Dr. med. Otmar Trentz, Director Clinic of Trauma Surgery
bis 31.8.2008

Prof. Dr. med. Hans-Peter Simmen, Director Clinic of Trauma Surgery
ab 1.10.2008

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

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

Prof. Dr. med. Michele Genoni Director Clinic of Cardiovascular Surgery

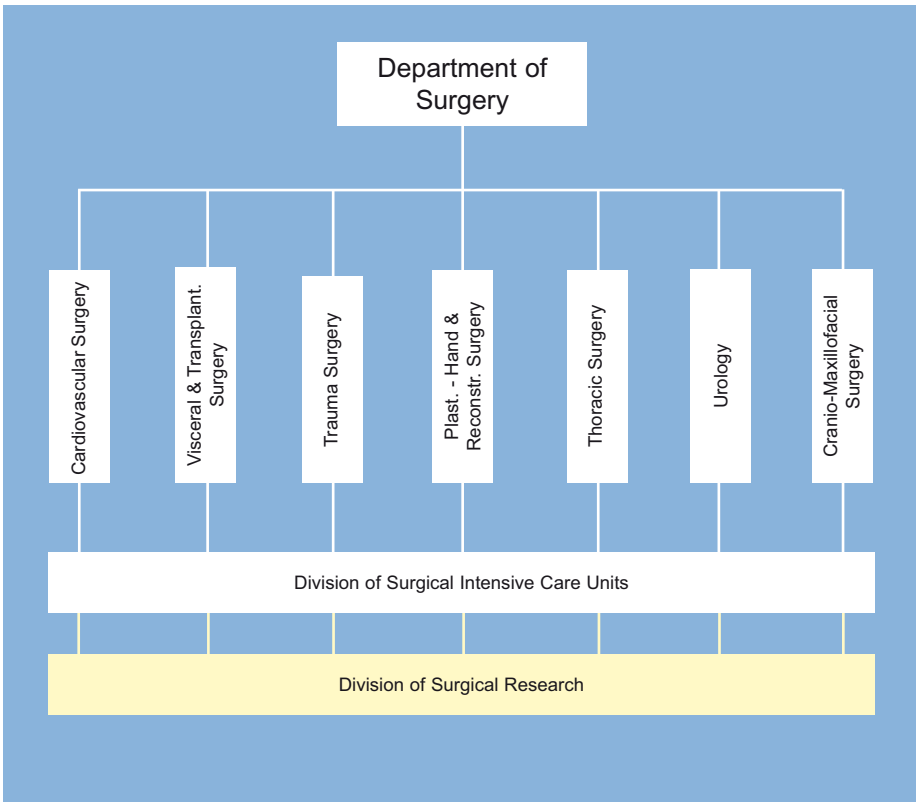
Prof. Dr. med. Pietro Giovanoli, Director Clinic of Plastic - Hand & Reconstr. Surgery

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

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

Prof. Dr. med. Reto Stocker, Head of Intensive Care Unit

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

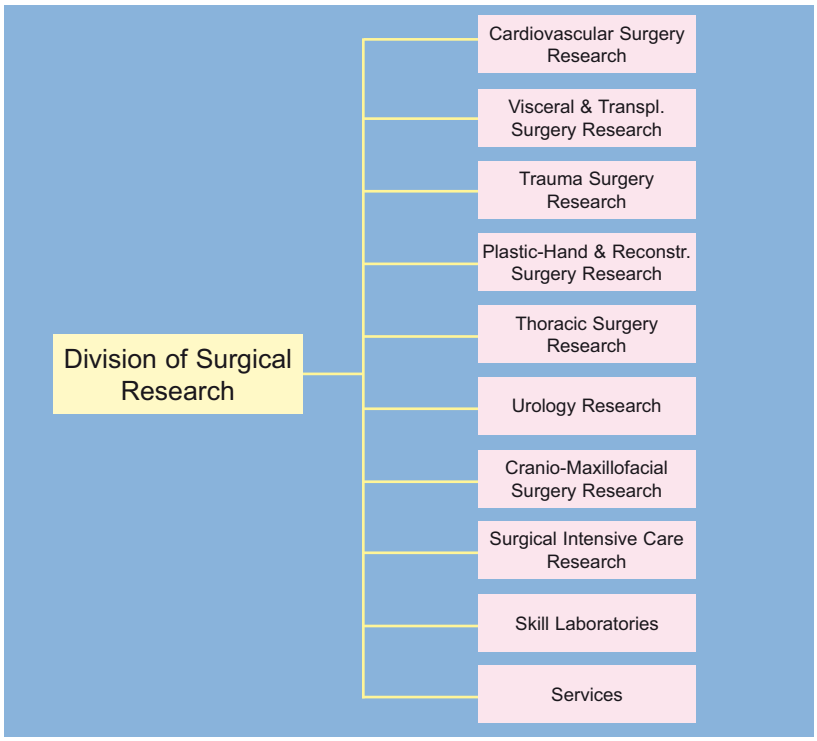


1.2 Structural Organisation of the Division of Surgical Research

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

PD Dr. phil. II
Rolf Graf,
Co-Head Division of
Surgical Research

Susanne Frehner,
Administration
Division of Surgical
Research

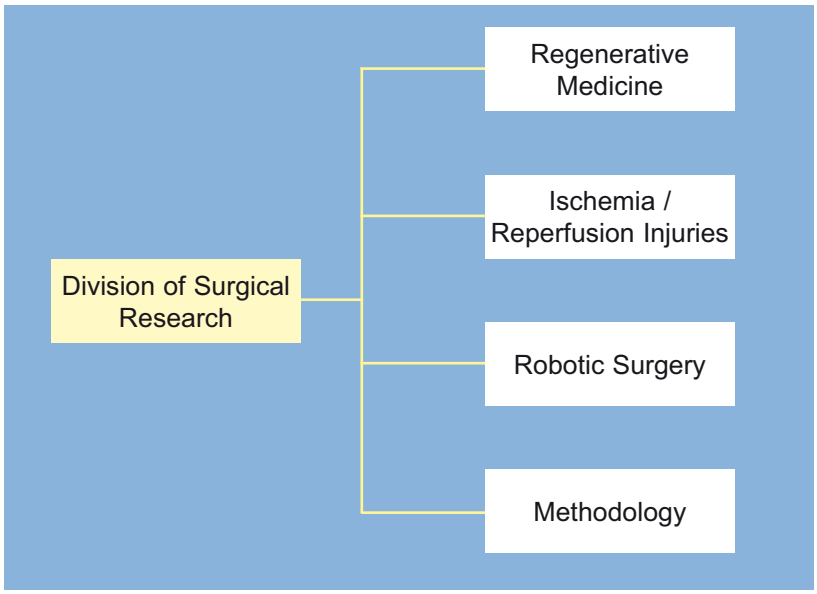


1.3 Scientific Sections within the Division of Surgical Research

Prof. Dr. med.
Simon Philipp
Hoerstrup
Regenerative
Medicine

Dr. phil II
Wolfgang Moritz
Ischemia /
Reperfusion
Injuries

PD Dr. phil II
Rolf Graf
Methodology



2. Research and Development

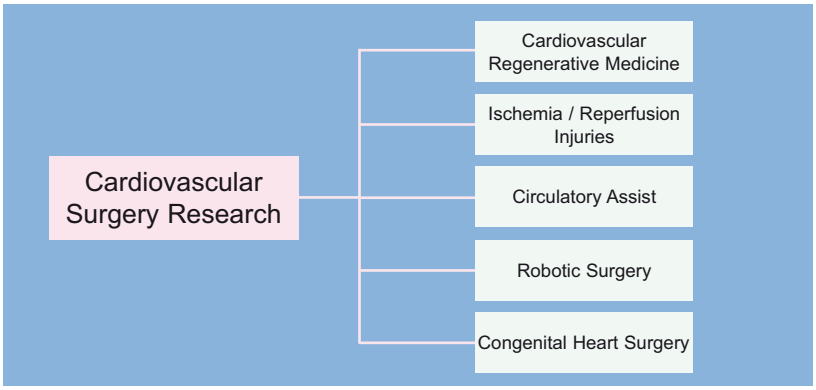
2.1 Cardiovascular Surgery Research


Prof. Dr. med.
Simon Philipp
Hoerstrup


Prof. Dr. med.
Michele Genoni


Sandra Edwin
Study Coordination
and Administration


Anika Lewandowski
Study Coordination
and Administration




Prof. Dr. med.
Dr. rer. nat. Simon
Philipp Hoerstrup


Dr. med.
Dr. rer. nat.
Dörthe Schmidt


Dr. sc. nat.
Jens Kelm


Dr. sc. nat.
Irina Agarkova


Dr. sc. nat.
Ronan Schönauer


Dr. med.
Christian Schmidt


Rene Stenger
Bachelor
Chemistry


Dr. med.
Alberto Weber


cand. med.
Armin Zürcher


cand. med.
Silvan Holdener


cand. med.
Sandro Imbach


Master Student
Volker Lorber


cand. med.
Boris Jenni


Master Student
Sarah Ronken


cand. med.
Michael Bullen

2.1.1 Cardiovascular Regenerative Medicine (Tissue Engineering and Cell Transplantation)

Prof. Dr. med. Dr. rer. nat. Simon Philipp Hoerstrup
(Head, Regenerative Medicine Program)

The Cardiovascular Regenerative Medicine Program comprises Tissue Engineering and Cell Transplantation and is focused on the development and in vitro generation of novel, cell based therapies for cardiovascular applications. These include tissue engineered blood vessels, heart valves as well as microscale strategies for myocardial regeneration. Presently 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 growth for congenital application as an alternative to state of the art artificial replacements. Furthermore, an additional focus is the development of cell based implants based on the design of in vitro generated microtissues to improve myocardial functionality of the diseased heart.

Research projects:

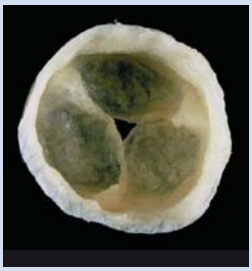
- Human Cell-Based Systems (progenitor, fetal, adult)
- Extracellular Matrix (proteins, tensegrity)
- Biomaterials (biodegradable, intelligent material systems)
- Bioreactor Systems
- Biomechanics, Computational Models, Molecular Imaging
- Animal Models (small and large)
- Tissue Engineered Cardiovascular Structures (Heart Valves, Vascular Grafts)
- Microtissue-Based Implants (Myocardium) and Cell Transplantation
- Molecular Imaging

cand. med.
Chad Brokopp

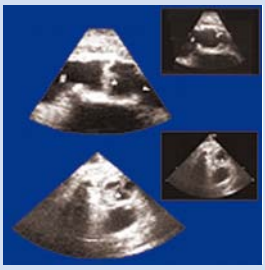
cand. med.
Pascal Heye

cand. med.
Karim Saba

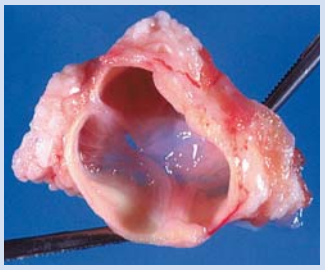
Proof of Heart Valve Tissue Engineering Concept



pre-implantation



in-vivo

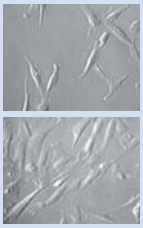


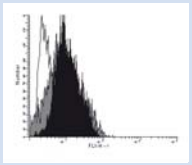
post-explantation

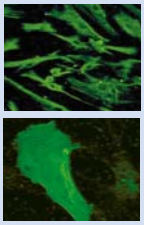
Autologous living tissue engineered heart valve in a sheep model, based on vascular - derived myofibroblasts and endothelial cells

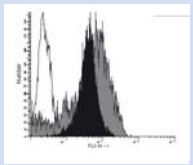
Hoerstrup et al. Circulation 2000

Human Heart Valve Tissue Engineering









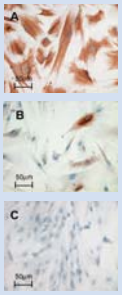
Heart valve tissue engineered from human marrow stromal cells

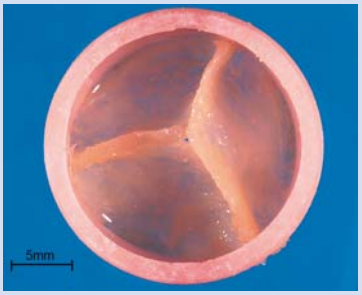
Hoerstrup et al. Circulation 2002

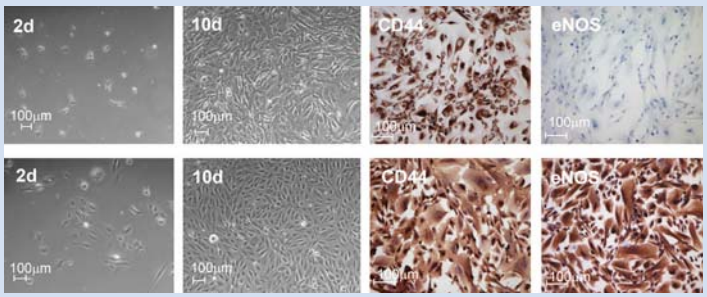
Human Prenatal Stem Cells for Pediatric Cardiovascular Tissue Engineering

Differentiated human chorionic villi-derived prenatal progenitor cells demonstrated phenotypes similar to interstitial cells of native heart valves by expressing vimentin (A) and partly a-SMA (B) and a lack of desmin (C) and could be successfully used for the fabrication of autologous heart valves.

Schmidt D et al. Circulation 2006







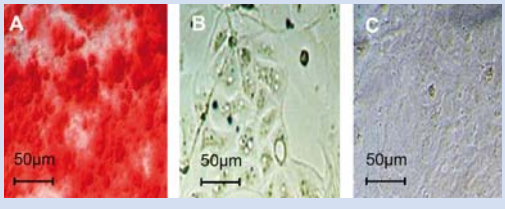
CD133 - cells

CD133 + cells

Fetal amniotic fluid-derived CD133+ and CD133- cells for autologous peditaric heart valve tissue engineering.
Schmidt D et al. Circulation 2007

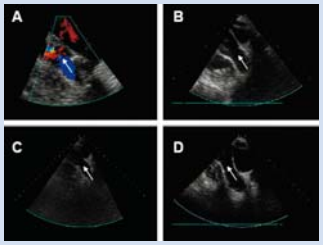
Differentiation potential of cryopreserved fetal amnitic fluid-derived cells: (A) When exposed to osteoblast-inducing medium production of calcium could be detected in alizarin red staining (A). In response to adipocytic stimuli vesicles appeared in Oil-Red-O staining (B) compared to cells cultured in α-MEM only. .

Schmidt D et al. J Heart Valve Disease 2008

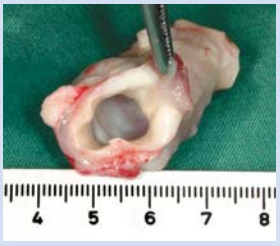


Functional Growth in Living Cardiovascular Grafts

pre-implantation



in-vivo

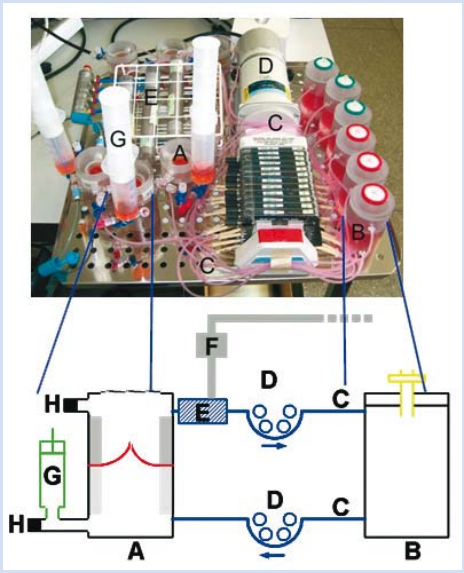


post-explantation

Autologous living tissue engineered pulmonary arteries demonstrated growth characteristics in a sheep model follwed up to 100 weeks.

Hoerstrup SP et al. Circulation 2006

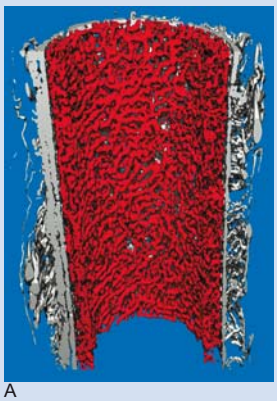
Bioreactor Development



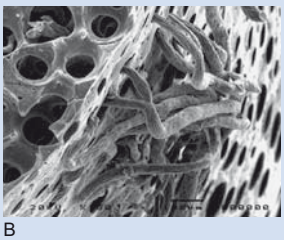
- sized small
- biocompatible materials
- maintenance of sterility
- static conditioning by continuous perfusion (4ml/min)
- dynamic conditioning by mimicking diastolic phase

Mol et al. Ann Biomed Eng 2005

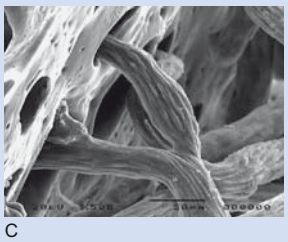
Neovascularisation of Biomaterials through Growth Factor Delivery



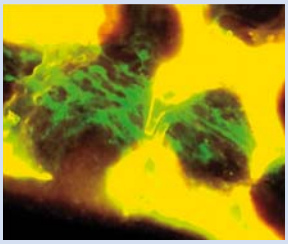
A



B



C

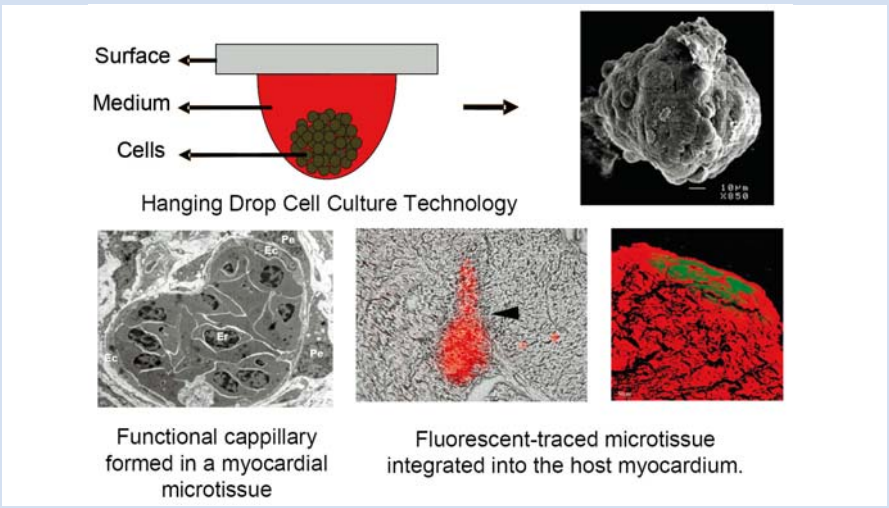


D

Increased Neovascularisation of porous Polyurethane as future bypass graft material induced by Vascular Endothelial Growth Factor (VEGF). Quantification of micro vascular network by Micro-CT (A), Corrosion Casting (B, C) and Lectin Perfusion (D)

(*Schmidt D et al.*) *unpublished data*

Design of Microtissues for Myocardial Regeneration

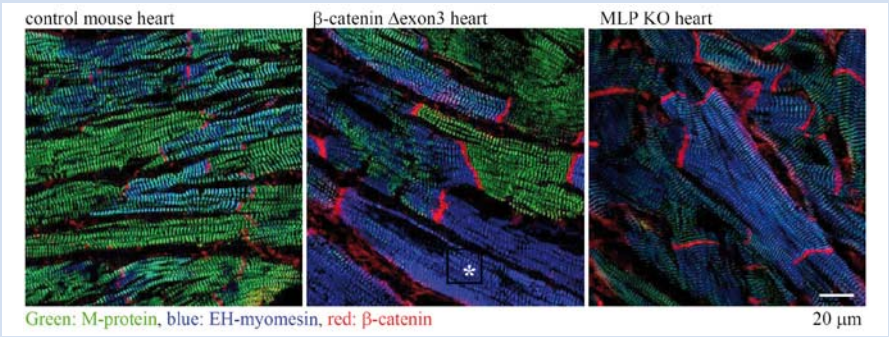


Myocardial microtissues are generated by gravity-enforced assembly of monodispersed cardiomyocytes in hanging drops. Myocardial microtissues (i) retain heart-muscle-specific morphology, (ii) express extracellular matrix components (iii) induce neovascularization, and (iv) integrate seamlessly into the myocardium after implantation into adult rats. Therefore they represent an attractive implantation format for cell-based therapy.

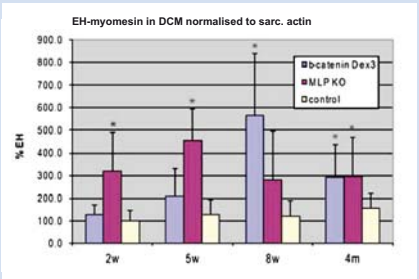
Kelm et al. Tissue Engineering 2006

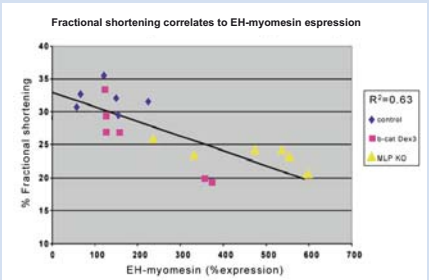
Characterisation of sarcomeric alterations in dilated cardiomyopathy

Immunofluorescent staining of heart cryosections of two transgenic mouse models (MLP KO, β-catenin Δexon3), which progressively develop dilated cardiomyopathy (age: 4 months).



The diseased hearts are characterised by size irregularities of the cardiomyocytes and higher heterogeneity of the cytoskeletal components. Some cells change the M-band protein expression to a completely embryonic phenotype (, blue staining). In addition, intercalated disk proteins (e.g. β-catenin in red) are upregulated in dilated cardiomyopathy models.*





The EH-myomesin isoform is significantly upregulated in both transgenic mouse models for dilated cardiomyopathy (DCM, left graph). The expression level of this embryonic heart specific isoform correlates to the degree of cardiac disease (% fractional shortening at the age of 5 weeks, right graph).

Schoenauer et al., unpublished data

Influence of nano-currents on cells viability

A plethora of bioelectrodes currently used in biomedical and bioengineering applications have difficulty producing consistent and stable recordings because of the biological response mounted against the implanted electrodes. The main cause of this is an increase in electrode impedance as a result of cell adhesion to, and the formation of a fibrous tissue matrix around the electrodes. This study demonstrates the use of a 50% square wave output signal of between 30nA-3μA to induce cell death in a highly localised area. Rat aortic endothelial cells (RAOEC) were seeded directly onto custom made electrodes for a range of time periods.

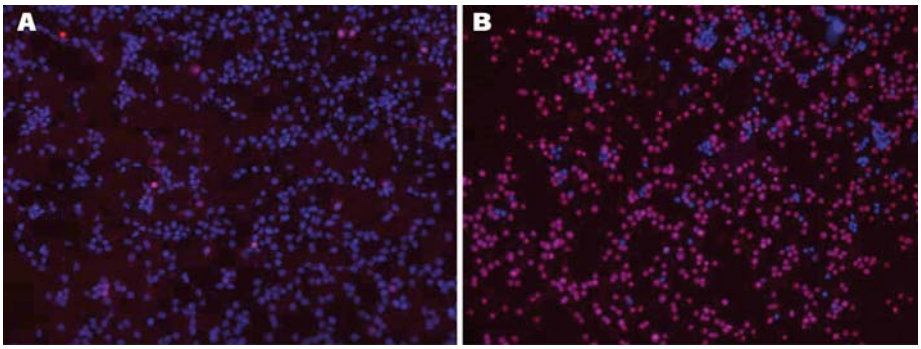


Figure 1: Cells seeded onto electrodes were stained with propidium iodide (red) to indicate membrane rupture and therefore death, and counterstained with DAPI to show living cells (blue). A) Control electrode without current after twelve hours. B) Working electrode after twelve hours)

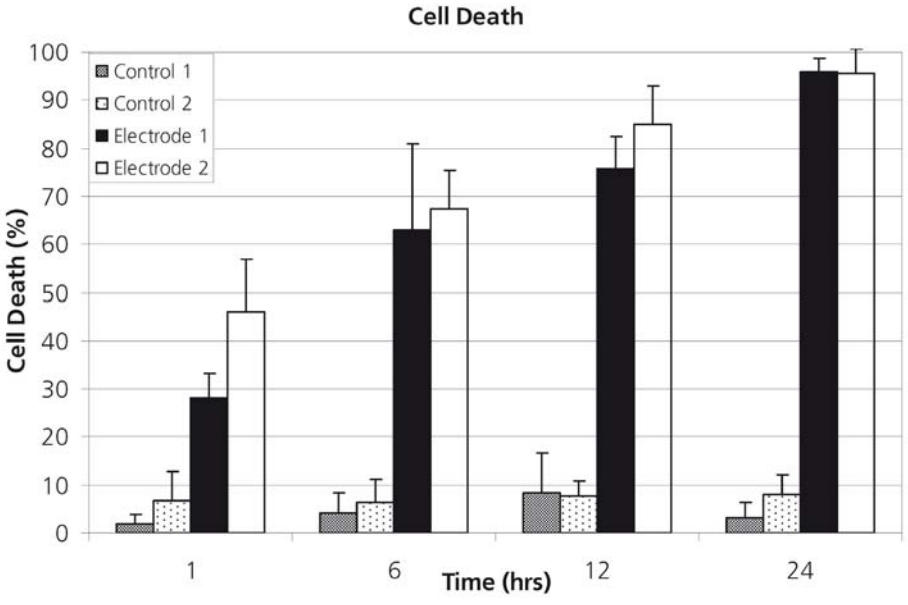


Figure 2: Summary of quantitative assessment of cell death

Achievements 2008

- Research grant: „Sarcomeric M-band as a novel marker for the remodeling process in cardiomyopathy" Foundation for Research at the Faculty of Medicine, University of Zurich. R. Schoenauer, 2008 – 2010.
- Research grant: „Sarcomere remodelling in the failing heart: implications for the disease mechanism." Roche Research Foundation. I. Agarkova, 2008 – 2009.
- Schmidt D. 7th Day of Clinical Research, Zurich, Switzerland: Best Presentation 2008

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, Zürich,Switzerland
- Department of Biochemistry, University Zürich, Switzerland
- Department of Mathematics, Federal Institute of Technology, Zürich, Switzerland
- Department of Computational Science, Federal Institute of Technology, Zürich, Switzerland
- Department of Veterinary Surgery, MSRU Vetclinics, University Zürich, Switzerland
- Department of Cardiology, University Hospital Zürich, 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
- Laboratory for Transplantation Immunology, University Hospital Zürich, Switzerland
- Institute of Chemistry and Applied Biosciences, Federal Institute of Technology Zürich, Switzerland
- Institute of Anatomie, University of Bern, Switzerland
- Human Genetics Laboratory, Genetica AG, Zurich, Switzerland
- Department of Pathology, University Hospital, Zurich, Switzerland
- Randall Division of Cell and Molecular Biophysics, King's College London, UK

Selected references:

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- Schmidt D, Asmis LM, Odermatt B, Kelm J, Breymann C, Gossi M, Genoni M, Zund G, Hoerstrup SP. Engineered living blood vessels: functional endothelia generated from human umbilical cord-derived progenitors. Ann Thorac Surg. 2006 Oct;82(4):1465-71
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2.1.2 Ischemia / Reperfusion Injury

PD Dr. med.
Reza Tavakoli

Dr. rer. nat.
Anna Bogdanova

Dr. Deyan Mihov

Erythropoietin protects from reperfusion-induced myocardial injury by enhancing coronary endothelial nitric oxide production

PD Dr. med. R. Tavakoli, Dr. sc. nat. Inna Agarkova,
Dr. sc. ETH Roman Schönauer, Dr. rer.nat. A. Bogdanova, Dr. D. Mihov

Objective: Cardioprotective properties of recombinant human Erythropoietin (rhEpo) have been shown in in vivo regional or ex vivo global models of ischemia-reperfusion (I/R) injury. The aim of this study was to characterize the cardioprotective potential of rhEPO in an in vivo experimental model of global I/R approximating the clinical cardiac surgical setting and to gain insights into the myocardial binding sites of rhEpo and the mechanism involved in its cardioprotective effect.

Methods: Hearts of donor Lewis rats were arrested with cold crystalloid cardioplegia and after 45 min of cold global ischemia grafted heterotopically into the abdomen of recipient Lewis rats. Recipients were randomly assigned to control non-treated or Epo-treated group receiving 5000 U/kg of rhEpo intravenously 20 min prior to reperfusion. At 5 time points 5-1440 min. after reperfusion, the recipients (n=6-8 at each point) were sacrificed, blood and native and grafted hearts harvested for subsequent analysis.

Results: Treatment with rhEpo resulted in a significant reduction in myocardial I/R injury (plasma Troponin T) in correlation with preservation of the myocardial redox state (reduced glutathione). The extent of apoptosis (activity of caspases 3 and 9, Tunel test) in our model was very modest and not significantly affected by rhEpo. Immuno-staining of the heart tissue with anti-Epo antibodies showed an exclusive binding of rhEpo to the coronary endothelium with no binding of rhEpo to cardiomyocytes (Figure 1 A: after 5 min and B: after 30 min of reperfusion).

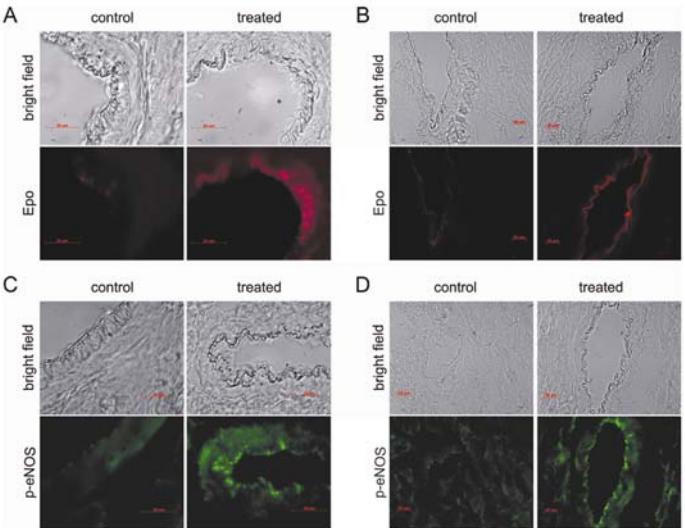

Figure 1

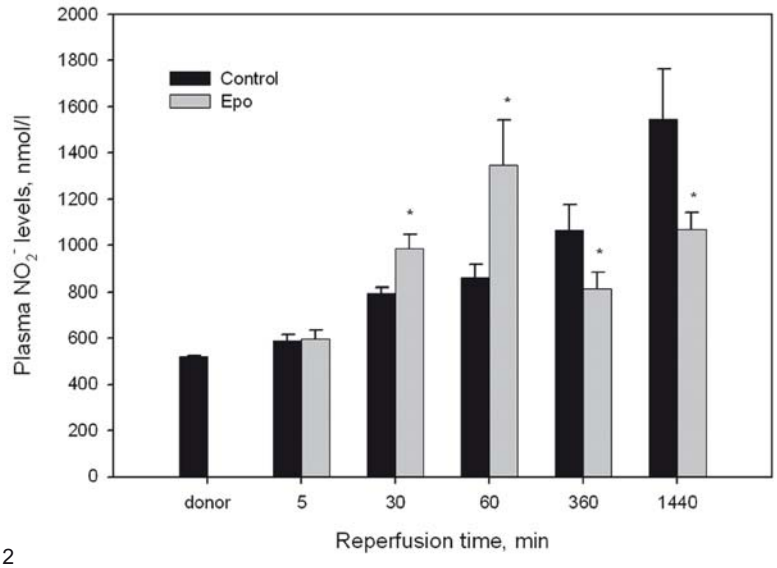

Figure 2

Administration of rhEpo resulted in a significant increase in nitric oxide (NO) production assessed by plasma nitrite levels (Figure 2). Immuno-staining of heart tissue with anti-phospho-eNOS antibodies showed that after binding to the coronary endothelium, rhEpo increased the phosphorylation and thus activation of endothelial nitric oxide synthase (eNOS) in coronary vessels (Figure 1 C: after 5 min and D: after 30 min of reperfusion). There was no activation of eNOS in cardiomyocytes.

Conclusions: Intravenous administration of rhEpo protects the heart against cold global I/R. Apoptosis does not seem to play a major role in the process of tissue injury in this model. After binding to the coronary endothelium, rhEpo enhances NO production by phosphorylation and thus activation of eNOS in coronary vessels. Our results suggest that cardioprotective properties of rhEpo are at least partially mediated by NO released by the coronary endothelium.

Achievements 2008

- A Bogdanova, D Mihov, G Zund, M Gassmann, R Tavakoli
  Mechanism of cardioprotective properties of erythropoietin during cold global ischemia and reperfusion
  Oral Presentation, 22d Annual Meeting of the European Association for Cardio-thoracic Surgery, September 2008, Lisbon, Portugal
- Bogdanova A, Mihov D, Bogdanov N, Gassmann M, Tavakoli R
  Characterization of erythropoietin interaction with myocardial tissue
  PosterAward, 4th ZIHP Annual Meeting, August 2008, Zurich
- Mihov D, Bogdanov N, Grenacher B, Gassmann M, Zünd G, Bogdanova A, Tavakoli R
  Erythropoietin protects from reperfusion-induced myocardial injury by enhancing coronary endothelial nitric oxide production.
  Eur J Cardio-thorac Surg, in press

Collaborations:

- Dr L Bestmann, Institute for clinical chemistry, University hospital Zurich
- Institute of Veterinary Physiology, Vetsuisse Faculty, University of Zurich
- Center of Integrative Human Physiology, University of Zurich
- Institute of Veterinary Physiology, Vetsuisse-Faculty University of Zurich

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2.1.3 Mechanical Circulatory Support

PD Dr. med.
Mario Lachat

Prof Dr. med.
Rene Pretre

PD Dr. Mario Lachat, Prof. Dr. R. Prétre

Long-term support

For left ventricular support, the Berlin Heart INCOR, a magnetically suspended and intracorporeally implanted axial-flow pump for left ventricular support, was used. Until end of 2008, 15 patients were supported with this device (fig. 1). Eight patients were transplanted successfully, and two patient were switched to a biventricular device. A young mother of two kids could be weaned from the device. Four patients died. Eight patients could be treated as outpatients, and three patients went back to work while being on support.
The Berlin Heart EXCOR is an extracorporeally located pulsatile pump (fig. 2). It is used for biventricular or right ventricular support. Until end of 2008,19 patients were supported with the EXCOR. Eleven patients were discharged home with the device during the waiting time for heart transplantation, two of which went back to work and school, respectively. Ten patients were transplanted, one could be weaned, four died, and four are currently still on support.

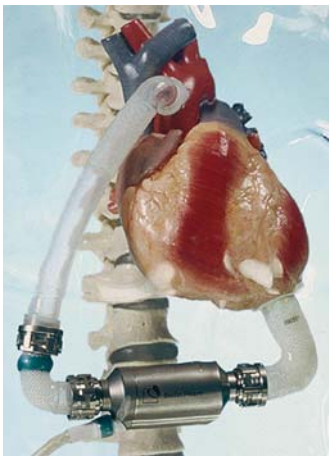


Figure 1 Berlin Heart INCOR (intrakorporale Lage)

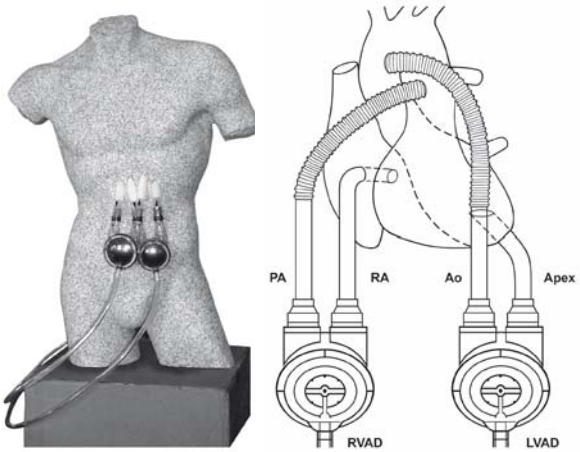
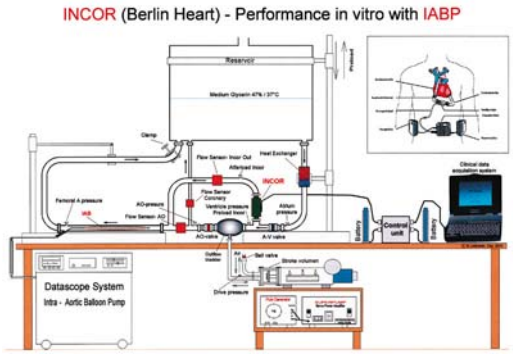


Figure 2 Berlin Heart EXCOR (links: extrakorporale Lage, rechts: Implantationsprinzip)

Short-term support

Short-term support In acute heart failure, veno-arterial ECMO (extracorporeal membrane oxygenation) was implanted in patients with postcardiotomy heart failure, and as rescue therapy in patients with rapidly developing cardiogenic shock as bridge to long-term mechanical support or transplantation. In lung failure, veno-venous or veno-veno/arterial ECMO was implanted in patients with ARDS due to causes such as fulminant pneumonia. In 2008, 20 such patients were supported with the ECMO. ECMO support extended up to 4 weeks with good mechanical reliability.



Achievements 2008

- Mechanical circulatory support program with excellent bridge-to-transplant rate
- Successful outpatient program of Assist-Device-Patients
- Reintegration of Assist-Device-Patients into working life

Collaborations:

- Levitronics Inc. (Zurich and Boston, USA)
- Berlin Heart (Berlin, Germany)

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2.1.4 Robotic Surgery and Innovative Technologies

PD Dr. med.
Jürg Grünenfelder

Dr. med.
André Plass

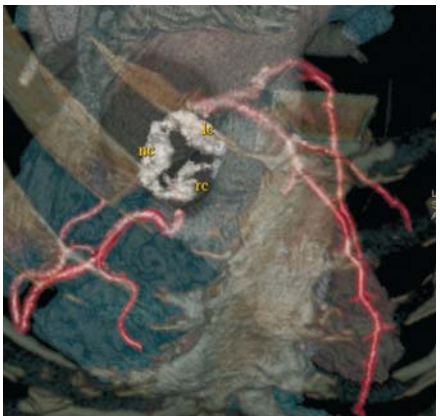
Dr. med.
Sacha Salzberg

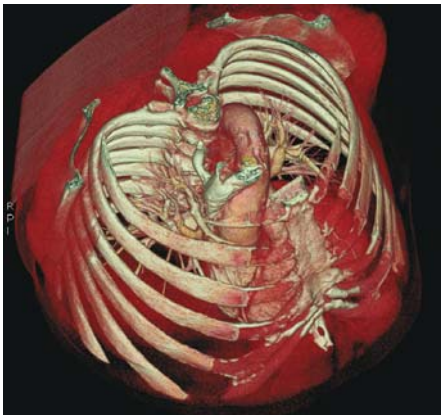
Minimally invasive cardiac surgery

PD Dr. med. Jürg Grünenfelder, Dr. med. André Plass,
Dr. med. Sacha Salzberg

- Automated coronary artery bypass operation in the beating heart
- Visualization and computational flow simulation in coronary arteries and through heart valves

Minimally invasive surgery (MIS) approaches have revolutionized surgery in the past decade, dramatically reducing morbidity and time required for healing and rehabilitation. Of the numerous barriers to more widespread implementation of MIS, probably the most significant is the problem of visualization. This general need to visualize the surgical site then relates to the needs for modelling patients, planning procedures, registering these plans to the patient, and guiding the surgeon in real time. Surmounting these barriers will open the way to MIS in a wide variety of new procedures.
The focus of this laboratory is the development of precise, adaptable, and widely deployable computer-integrated systems and devices that make a wide variety of surgical interventions newly amenable to minimally invasive cardiac surgical approaches by presenting minimally invasive tools as well as information about preoperative plans through simulation, anatomy, tool position and surgical progress to the surgeon in an ergonomic fashion.





Collaborations:

- Department of Radiology, Universtiy Hospital Zürich (Hatem Alkadhi, MD)
- Laboratory for Thermodynamics in Emerging Technologies, ETH Zürich (Prof. Dimos Poulikakos)

Selected references:

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2.1.5 Congenital Heart Surgery

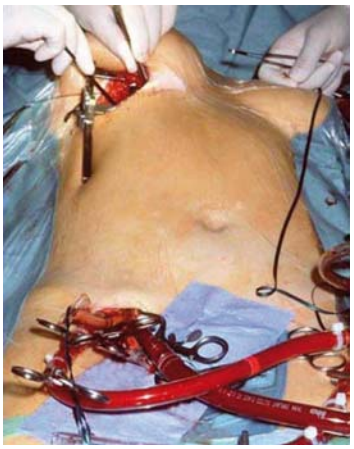
Prof Dr. med.
Rene Pretre

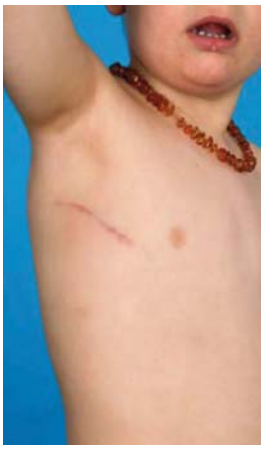
Dr. med.
Hitendu Dave

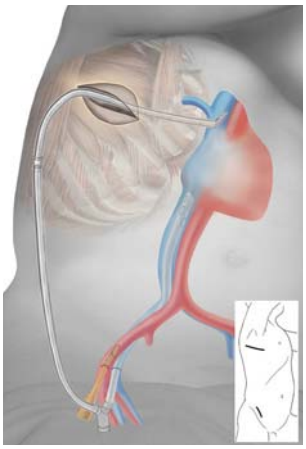
Prof. Dr. med. René Prêtre, Dr. med. Hitendu Dave

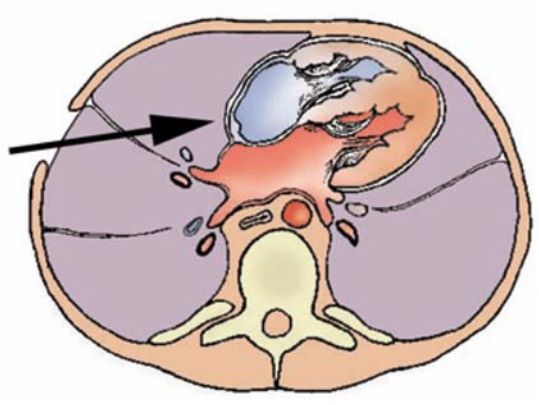
Projects

The Division of Congenital Cardiovascular Surgery at the University Children's Hospital is pursuing its efforts towards minimal invasiveness, both with regards to cosmetic mini-approaches, and the development of surgical instruments to achieve this goal.









Selected Projects

Analysis and condition of recovery of arteries and veins after short term banding: Classical technique of banding involves use of a Teflon band which is gradually tightened around the pulmonary artery. In order to simplify the procedure and standardize the end diameter after banding (without causing trauma) the Division of Congenital Cardiovascular Surgery has developed a device which can be clipped around the pulmonary artery.

Influence of extra-pleural approach in reducing formation of systemic to pulmonary artery collaterals in Univentricular hearts. This study tests a new hypothesis about the advantage of extra-pleural approach while performing operations on and around the aortic isthmus. Having pioneered this approach for performing resection of aortic coarctation and extended end-to-end anastomosis at our unit at Kinderspital Zurich, it has been observed that the development of lung parenchyma to thoracic wall collaterals are minimized by maintaining the pleural integrity. This study evaluates the formation of various types of collaterals in patients with univentricular hearts, who had undergone a thoracotomy for any reason and tries to compare the type and incidence of collaterals after a trans-pleural approach versus an extra-pleural approach.

Development of a new electrode for long- term intramyocardial ventricular stimulation: We evaluate a newly designed electrode that would be inserted directly into the myocardium avoiding the difficult positioning on the heart surface.

Development of minimal invasive techniques and devices for the correction of congenital cardiac malformations in children: The Division of Congenital Cardiovascular Surgery developed an “ecarteur thoracique” which can be used to improve the operating field in congenital heart surgery.

Achievements 2008

- Successful expansion of mini-thoracotomy approaches (repair of congenital heart defects: atrial septal defect, ventricular septal defect, partial anomalous pulmonary venous return)
- Implantation of Pacemaker electrodes and generator through muscle-sparing left axillary mini-thoracotomy
- Establishment and participating in several humanitarian projects in the so called „third world“

Collaborations:

- Division of Pediatric Cardiology, University Children’s Hospital, Zurich, Switzerland
- Department of Biostatistics, Institute for Social and Preventive Medicine, University of Zurich, Zurich, Switzerland
- International Childrens Heart Foundation, Russland, Sibirien, Kemerovo
- Biologisch Zentral Labor, University Hospital, Zurich, Switzerland
- European Association of CardioThoracic Surgery Congenital Database, Warsaw, Poland
- Berlin Heart, Berlin, Germany

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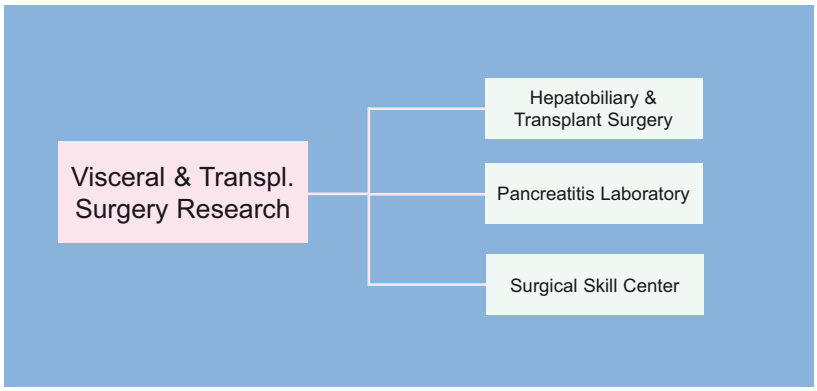
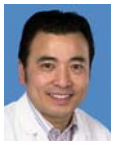
2.2 Visceral & Transplant Surgery Research

PD Dr. phil II
Rolf Graf

Prof. Dr. med.
Pierre-Alain Clavien



2.2.1 Hepatobiliary & Transplant Surgery



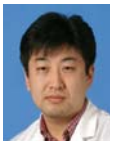
PD Dr. med.
Yinghua Tian

Dr. sc. nat.
Wolfgang Moritz

Dr. med.
Andreas Rickenbacher

Dr. med.
Ashraf Osman

Dr. med.
Katarzyna Furrer



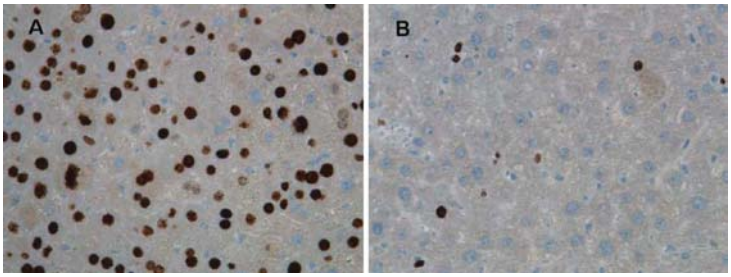
Dr. sc. nat.
Jae-Hwi Jang

Dr. med.
Kuno Lehmann

Udo Ungethüm

Liver Injury and Regeneration
Cholestasis inhibits liver regeneration
Dr. med. Andreas Rickenbacher

Patients with cholestasis that need a liver resection due to tumors have a worse postoperative clinical outcome compared to non cholestatic patients. The reason with its underlying molecular mechanisms is not known in detail and is therefore evaluated in our lab.
Basically two reasons could be responsible for this effect. During the liver resection the pringle-maneuver is often applied to avoid blood loss and therefore an ischemia/reperfusion (I/R) injury inevitably occurs. It is likely that more damage occurs during I/R in cholestasis. In a previous publication from our lab we evaluated this question and we could not only show that cholestasis does not harm but that it is actually protective during I/R.
The other possibility is an impaired liver regeneration that could be inhibited by cholestasis. Many growth factors and cytokines are involved in the process of liver regeneration. They are closely regulated and allow the liver to regenerate and reach the majority of its original weight within a few days.
To answer the question whether liver regeneration is impaired, we developed a cholestatic mouse model by ligating the common bile duct followed by a partial hepatectomy seven days after the bile duct ligation. The proliferation indexes are significantly downregulated when cholestasis is present.

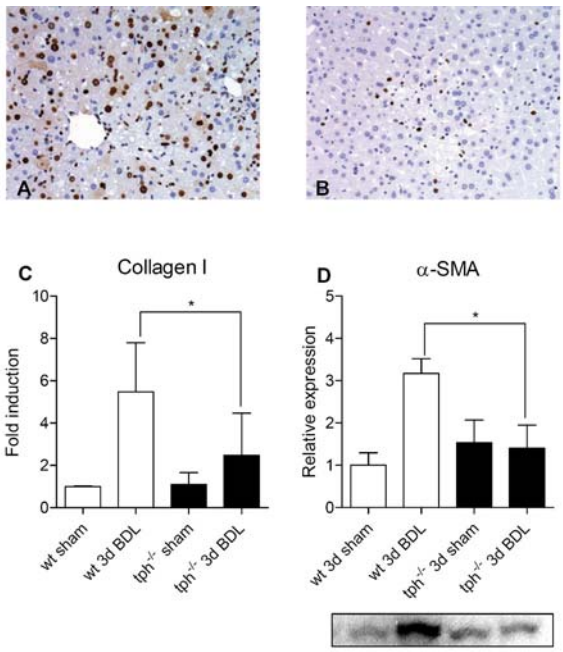


Ki-67-immunhoistochemistry of control (A) and cholestatic livers (B) 48h after partial hepatectomy show normal regeneration in control animals and impaired regeneration in the presence of cholestasis.

To clarify why cholestasis inhibits hepatocyte proliferation we study this phenomenon in more detail. Inhibitory cytokines of the TGFβ-superfamily are possible candidates as those are upregulated in cholestasis.

Serotonin mediates wound healing in a mouse model of acute liver injury
Dr. Jae-Hwi Jang, Dr. med. Andreas Rickenbacher, Dr. med. Panco Georgiev, Dr. med. Christopher Soll

Serotonin, a monoamine neurotransmitter stored in platelets, is required for liver regeneration after major liver resection. Since tissue repair after hepatic injury may also be affected by serotonin we asked whether serotonin contributes to hepatic wound healing after acute liver injury. Hepatic injury was induced by total bile duct ligation (BDL) or CCl4 injection for 1, 3, 14, and 42 days in wild type (wt) and tryptophan hydroxylase 1 (tph) knockout mice, lacking peripherial serotonin. Hepatic injury was shown by the liver enzymes AST and ALT, and morphometric evaluation of the necrotic area. Markers of liver fibrosis, such as collagen I, α-smooth muscle actin (SMA), and transforming growth factor β-1 (TGF β-1) were assessed by RT-PCR and western blotting. Fibrogenesis was also studied in immune-thrombocytopenic wild type mice induced by intra-peritoneal injection of CD41 antibody. At 3 days after BDL, fibrosis was more pronounced in wild type mice as compared to tph (-/-) or immune-thrombocytopenic mice, characterized by increased expression levels of collagen and α-SMA. In contrast, liver injury as assessed by serum AST and ALT levels was significantly higher in tph (-/-) mice. Ki-67 staining showed considerably higher proliferative activity of hepatocytes in wt mouse livers after 3 days of BDL. This data provides evidence that platelet-derived serotonin contributes to acute wound healing including fibrogenesis and regeneration.



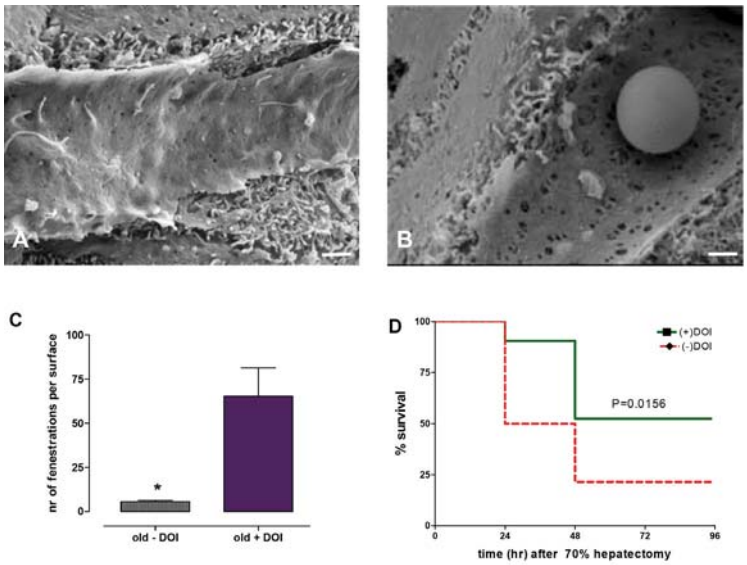
The regenerative response of mouse livers 3 days after bile duct ligation assessed by Ki67 staining: (A) wild type mouse, (B) tph (-/-) mouse. Magnification 200x. Expression of (C) collagen I mRNA and (D) SMA protein in wild type mice and tph (-/-) mice measured in livers at 3 days after bile duct ligation (BDL). The extent of fibrosis is significantly reduced in mice lacking peripherial serotonin. *p<0.05. n=4

Serotonin enhances sinusoidal endothelial cell fenestration and improves liver regeneration in old mice

Dr. med. Katarzyna Furrer, PD Dr. med. Yinghua Tian

The increasing age of patients with liver disease requiring surgery leads to more complications related to failure of regeneration. Previous work from our group demonstrated that the aging liver has a reduced capacity to regenerate after major tissue loss. In addition to molecular changes, it has been observed that the sinusoidal structure is severely affected in older individuals. A loss of fenestration in the sinusoidal endothelial cells (SEC) might reduce the flow of blood components and soluble mediators into the space of Disse resulting in impaired signalling. Serotonin has recently been implicated in the early process of regeneration in young mice. We therefore hypothesize that serotonin may influence fenestration and propagate access to the parenchyma after tissue loss. Young (3 months) and old mice (2 years) underwent 70% partial hepatectomy. A serotonin receptor agonist, DOI, was used to pretreat mice two days before hepatectomy.

In contrast to young mice, the SEC of old mice exhibited hardly any fenestrae. Consistent with these changes, regeneration was impaired in old mice. During regeneration, the expression of serotonin receptor mRNA was highly increased in young but not in old livers. Pre-treatment of old mice with a serotonin receptor agonist partially reverted fenestration of SEC similar to the young phenotype. Subsequently, hepatectomy in the DOI treated old mice disclosed improved regeneration as demonstrated by increased numbers of proliferating hepatocytes. Furthermore, these animals had a better survival after hepatectomy. We conclude that serotonin improves regeneration in old mice by increasing SEC fenestration. These results may open the path for pharmacological targeting of serotonin receptors in the liver and may provide a simple approach to improve surgical interventions in the aging population.



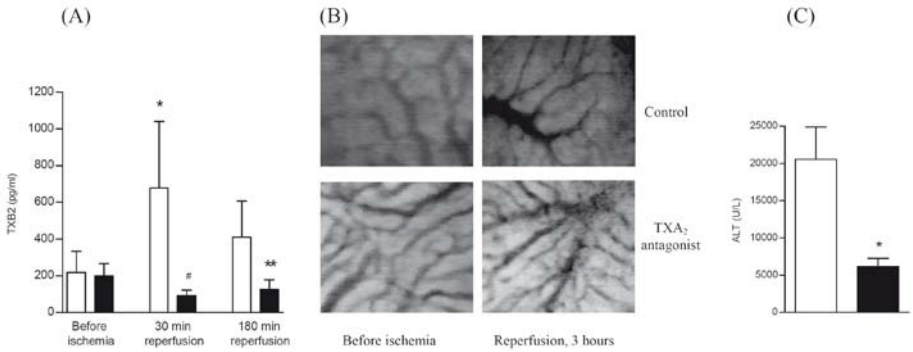
(A) Scanning electron microscopy reveals classical membrane thickening and lack of fenestrae in sinusoidal endothelial cells in livers of old mice (18-24 months). Bar=1µm. (B, C) Treatment with the 5HT2 receptor agonist DOI led to a dramatic increase in fenestrae formation and (D) survival after 70% partial hepatectomy. *p<0.001, n=4

Thromboxane A2 Mediates Ischemia/Reperfusion Injury of the Steatotic Mouse Liver

Dr. med. Ashraf Mohammad El-Badry, Dr. Jae Hwi Jang, PD Dr. med. Yinghua Tian

Thromboxane A2 (TXA2) is a potent vasoactive eicosanoid derived from arachidonic acid (AA) which belongs to omega-6 fatty acids (n-6 FAs). We hypothesized that increased TXA2 synthesis due predominance of n-6 FA enhances the susceptibility of the steatotic liver to ischemia/reperfusion (I/R) injury. Ob/ob mice were fed a fish oil enriched diet (source of n-3 FA) to decelerate the conversion of n-6 FA precursors to AA. Indeed, dietary n-3 FAs reduced the hepatic content of AA. In a model of partial hepatic ischemia, control diet fed animals showed increased TXA2 levels 30 minutes after reperfusion and a remarkable reduction of red blood cell velocity and volumetric blood flow in the suprahepatic vena cava. Supplementation with n-3 FAs consistently decreased TXA2 levels after reperfusion and ameliorated blood flow. In another set of experiments, the influence of TXA2 was abolished by a single intravenous bolus of selective TXA2 receptor antagonist. Concurrently, ALT levels disclosed pronounced reduction.

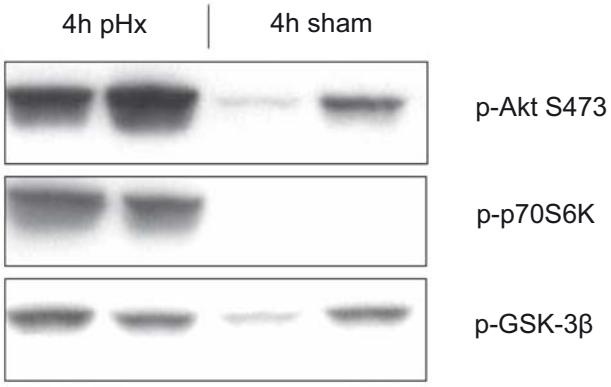
The reduced tolerance of the steatotic liver to I/R injury is explained, at least partially, by TXA2-mediated sinusoidal perfusion failure rather than an unproven lipid droplet-related mechanical effect. TXA2 blockage may rescue the steatotic liver from I/R injury after liver resection and could compensate for the shortage of donor organs for liver transplantation.



(A) Suprahepatic vena cava plasma levels of TXB2 (a metabolite of TXA2) before and after 45 min of ischemia in ob/ob mice fed with control diet (open bars) or with n-3 FA enriched diet (black bars). (B) Increased functional sinusoidal density before and after ischemia in livers from ob/ob mice treated with TXA2 antagonist. (C) Plasma ALT levels were significantly decreased 3 hrs after ischemia in ob/ob mice treated with TXA2 antagonist (black bar) compared to control (open bar). *p=0.024 compared to pre-ischemic levels. #p=0.007, **p=0.014 versus control diet. n=5

Akt isoforms in liver regeneration
Dr. med. Kuno Lehmann

The complex regulation of liver regeneration has many key players with yet unclear functions. Akt, also known as protein kinase B (PKB), is such a protein. Activation occurs by phosphorylation at two sites, downstream of growth factors or hormones. Currently, two isoforms of Akt (Akt1 and Akt2) are known to be present in the liver. A critical role in liver regeneration is highly suspected. However, the presence of two isoforms suggests an isoform-specific function during liver regeneration. After partial hepatectomy in mice, Akt phosphorylation showed an irregular pattern at different time points after hepatectomy and in control animals. However, four hours after partial hepatectomy, total phosphorylated Akt was significantly increased. Downstream targets of Akt, targeting protein synthesis (e.g. GSK3, p70S6 kinase), were also activated. Additionally, a cell cycle regulation protein was downregulated (p21). Based on these observations, we hope to identify an isoform specific function of Akt, regulating cell proliferation and cell growth during liver regeneration.



Phosphorylation pattern of AKT and its target proteins p70S6 kinase and GSK-3β in wild type mice at 4h after 70% of partial hepatectomy (pHx) in comparison to sham operated mice.

Achievements 2008

Findings:

- Liver regeneration is impaired in cholestatic mice.
- Serotonin mediates wound healing (fibrogenesis, liver regeneration) in a mouse model of acute liver injury.
- Livers from old mice show improved liver regeneration upon treatment with 5HT2 receptor agonist, possibly by enhanced sinusoidal endothelial cell fenestration.
- Thromboxane A2 antagonist treatment improves hepatic microcirculation and ischemic liver injury in obese mice.
- AKT signalling pathway is activated in a mouse model of partial hepatectomy.

Collaborations:

- Prof. Wolfram Jochum, PD Dr. med. Achim Weber, Institut für Klinische Pathologie, Universitätsspital Zürich
- Dr. med. Oliver Tschopp, Klinik für Endokrinologie and Diabetologie, Departement Innere Medizin, Universitätsspital Zürich
- Dr. Anne Greet Bittermann, Zentrum für Mikroskopie, Universität Zürich
- Dr. med. Claudio Contaldo, Dr. med. Ahmed Elsherbiny, Klinik für Wiederherstellungschirurgie, Universitätsspital Zürich

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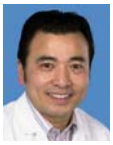


PD Dr. med.
Yinghua Tian

PD Dr. med.
Phillipp Dutkowski

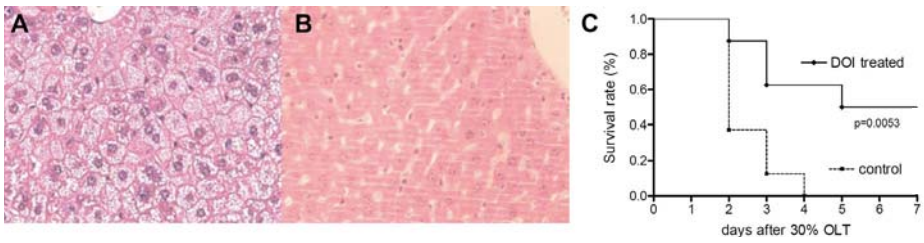
Dr. med.
Olivier de Rougemont

Liver Transplantation

Serotonin rescues small-for-size liver graft syndrome independently of IL-6 via HTR-2B pathway

PD Dr. med. Yinghua Tian

Living related liver transplantation (OLT) is a therapeutic option to circumvent the shortage of the liver donor pool. Unfortunately, this procedure is impeded by the small-for-size liver graft syndrome, which leads to impaired liver regeneration and graft failure after transplantation. We also have previously shown that liver regeneration depends on the presence of circulating serotonin. Consequently we aimed to investigate the role of serotonin in small-for-size OLT in mice. Small-for-size (30%) OLT were performed in wild type C57/B6 mice receiving saline or DOI, an agonist of serotonin receptor-2, which has been demonstrated to mediate serotonin dependent liver regeneration. The survival rate of DOI treated recipient animals was 50% compared with no survivals in the control group. The mRNA level of HTR2b (serotonin receptor subtype) was more than two-fold increased in the DOI group when compared to controls. H&E staining disclosed extensive microvesicular steatosis in hepatocytes of recipient control mice while this type of injury was significantly reduced in DOI treated animals. Ki-67 and PCNA staining showed significantly activated regeneration of hepatocytes in experimental recipients. AST levels at 2 days after 30% OLT were lower in DOI treated recipients compared to control animals. The beneficial effect of DOI was also observed in small-for-size (30%) OLT in IL-6-/- mice, suggesting that serotonin is acting independently of the IL-6 pathway. Improved liver function and recipient survival via the activation of the HTR-2B pathway provides a strong argument to target serotonin signalling as a therapeutic option to prevent the small-for-size liver syndrome.



Histology of small-for-size liver graft 3 days post Tx in (A) control (saline) and (B) DOI treated recipient mice. Note microvesicular steatosis in control treated mice which resolved upon DOI pretreatment. (C) Recipient survival after small-for-size liver transplantation in DOI treated mice in comparison to saline treated mice. n=8

Hypothermic oxygenated perfusion of non viable liver allografts donated after cardiac death

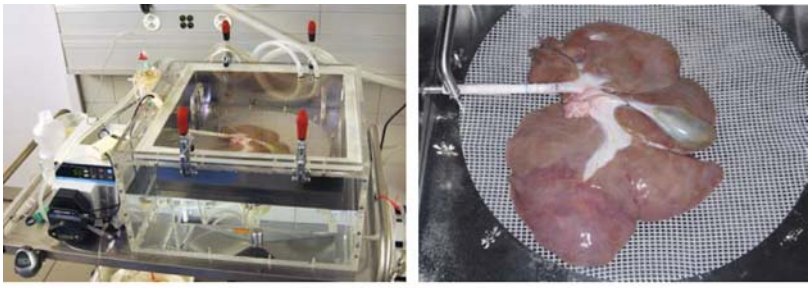
PD Dr. med. Philipp Dutkowski, Dr. med. Olivier de Rougemont

After having confirmed a strong protective effect of 1h HOPE (hypothermic oxygenated perfusion) ex vivo, we proceeded to the next experimental step and tested whether 1h HOPE would prevent injury in vivo. For this purpose, a new model of DCD (donation after cardiac death) liver transplantation in large pigs was developed.

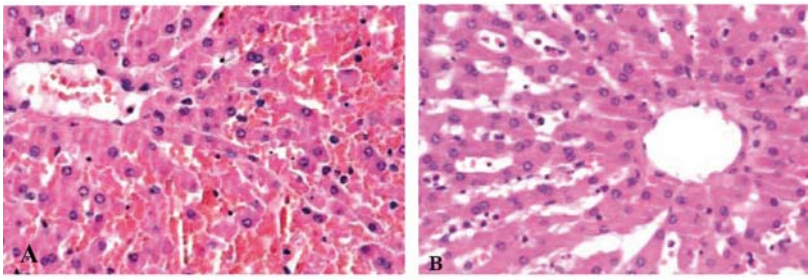
Pig livers were harvested 60 minutes after induction of cardiac death through asphyxia by withdrawal of the respirator. In situ flush and organ procurement were initiated without heparin pretreatment. After backtable preparation livers were preserved for 7 hours in cold Celsior (DCD-group) prior to orthotopic liver transplantation (OLT). Some livers were treated by one hour HOPE prior to implantation (HOPE-group). Animals were kept under anesthesia for 6 hours after OLT.

After 6 hours of in vivo reperfusion livers from the DCD group displayed macroscopically dark and inhomogeneous perfusion in contrast to HOPE treated grafts. Non treated DCD liver grafts showed increased adhesion of platelets, high AST (aspartate transaminases) release, absence of bile flow, depletion of glutathione and ATP. In contrast, livers treated with HOPE showed reduced platelet adhesion and decreased release of AST, while bile flow, ATP recovery, and glutathione were improved. Importantly, untreated DCD livers caused graft failure and death of recipients within 6h of reperfusion, whereas HOPE treated DCD livers remained hemodynamically stable.

We now attempt to extubate the animals after OLT to show viability of a DCD graft after HOPE treatment.



HOPE machine. Fully automated, pressure controlled, and sterilizable.



H&E staining of DCD grafts (A) without and (B) with HOPE treatment 6h after reperfusion

Achievements 2008

Findings:

- Serotonin ameliorates liver morphology and improves survival in a mouse model of small-for-size liver transplantation.
- Ex vivo hypothermic oxygenated perfusion (HOPE) improves graft function in a large animal liver transplant model.
- 20 orthotopic liver transplantations in the pig.
- Swiss Surgical Research Prize 2008: *Simple and effective machine perfusion of non heart beating donor pig livers before transplantation*. de Rougemont O, Dutkowski P, Furrer K, Graf R, Clavien P-A.

Collaborations:

- Boris Leskosek, Departement Chirurgie, Abteilung Forschung
- Flora Nicholls, Biologisches Zentrallabor, Universitätsspital Zürich

Selected references:

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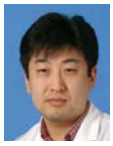
Dr. med.
Christopher Soll



Dr. sc. nat.
Jae-Hwi Jang

Oncology

Serotonin suppresses autophagy in human hepatocellular cancer cells

Dr. med. Christopher Soll, Dr. Jae Hwi Jang

Autophagy is a type of programmed altruistic cell death induced by withdrawal of nutrients. It is tightly regulated by specific proteins that promote assembly of autophagosomes and catabolize cytosolic components. Presumably, autophagy is a mechanism by which cells defend against temporal loss of nutrient supply resulting in either survival or controlled death. Nutrient depletion is also encountered by cancer cells which require continues angiogenesis to promote growth. A putative growth factor is serotonin, which supports liver regeneration and may also be involved in cancer growth. Two human hepatocellular carcinoma (HCC) cell lines were exposed to serotonin. No significant mitogenic activity was found, however, in serum-free medium, serotonin prevented cell death. Electron microscopy revealed a serotonin dependent inhibition of autophagosome formation. In the presence of rapamycin, an inhibitor of mTOR and inducer of autophagy, serotonin mediated phosphorylation of p70S6K and 4E-BP1, two downstream targets of mTOR that facilitate protein synthesis and cell cycle progression. Expression of the 5HT2B serotonin receptor and LC3, a marker for autophagy, was confirmed in human hepatocellular carcinoma. In a xenograft cancer model targeting the 5HT2B receptor decreased tumor growth. The study provides evidence that serotonin and autophagy are involved in the biology of hepatocellular cancer.

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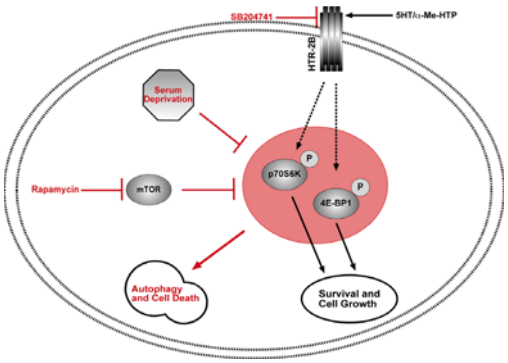

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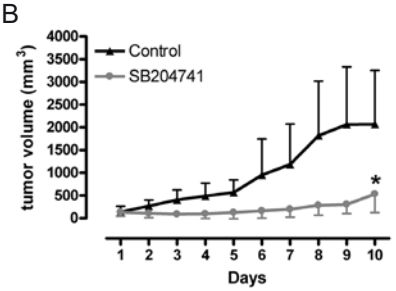

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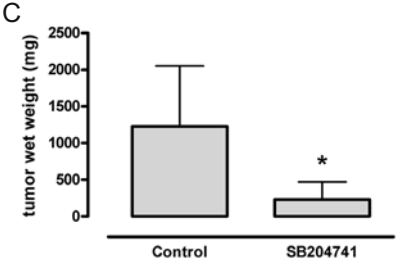

(A) Upon serum deprivation, human hepatocellular carcinoma cells (Huh7, HepG2) go through autophagy before cell death occurs. This effect can also be induced by inhibiting mTor activation by rapamycin and antagonizing the 5HT2B receptor by SB204741. Serotonin promotes cell survival under conditions of serum deprivation by activating p70S6 kinase and 4E-BP1 in a mTOR independent manner. (B) Tumor xenograft growth of Huh7 cells injected subcutaneously into nude mice is markedly decreased in mice treated with 5HT2R antagonist SB204741 when compared to control mice. n=6. *p=0.0055. (C) Tumor size assessed by weight after explantation. *p=0.0174

Achievements 2008

Findings:

- Serotonin inhibits autophagy and promotes cell survival in human hepatocellular carcinoma cells under serum deprivation.
- 5HT2R antagonist reduces xenograft tumor growth of Huh7 cells.

Collaborations:

- Dr. med. Marc-Oliver Riener and Peter Johannes Wild, Institut für Klinische Pathologie, Universitätsspital Zürich

Selected references:

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2.2.2 Pancreatitis Research Laboratory

PD Dr. phil. II
Rolf Graf

Dr. med.
Li-Kang Sun

Dipl. phil. II
Theresia
Reding Graf

Martha Bain

PD Dr. med.
Daniel Bimmler

Dr. sc. nat.
Alberto Silva

cand. med.
Soo-Young Kim

Pathophysiology pancreatitis
Dipl. phil. II Theresia Reding Graf, Dr. med. Li-Kang Sun, Martha Bain, PD Dr. R. Graf

The pathophysiology of human chronic pancreatitis is not well understood and difficult to follow on a molecular basis. Therefore, we used a rat model (WBN/Kob), which exhibits spontaneous chronic inflammation and fibrosis in the pancreas. We compared gene expression patterns in the pancreas during development of inflammation and fibrosis of WBN/Kob rats with age-matched healthy Wistar rats using microarrays. The extracellular matrix protein SPARC (secreted protein, acidic and rich in cysteines) and transcripts of inflammatory genes were quantified by real-time PCR and some localized by immunohistochemistry.

When pancreatic inflammation becomes obvious at the age of 16 weeks, several hundred genes are increased between 3- and 50-fold in WBN/Kob rats compared to healthy Wistar rats. Proteins produced by acinar cells and characteristic for inflammation, e.g. pancreatitis-associated protein (PAP), are highly upregulated. Other proteins, derived from infiltrating inflammatory cells and from activated stellate cells (fibrosis) such as collagens and fibronectins are also significantly upregulated. SPARC was localized to acinar cells where it increased in the vicinity of inflammatory foci. However, acinar expression of SPARC was lost during destruction of acinar cells. In human pancreatic specimens with chronic pancreatic inflammation, SPARC exhibited a similar expression profile.

During chronic inflammation and fibrosis in the WBN/Kob rat, inflammatory genes, growth factors and structural genes exhibit a high increase of expression. A temporal profile including pre- and post-inflammatory phases indicates a concurrent activation of inflammatory and fibrotic changes. Inflammation dependent expression of SPARC is localized in acinar cells and appears to be lost during acinar-to-duct metaplasia both in rat and human pancreas (Figure 1).

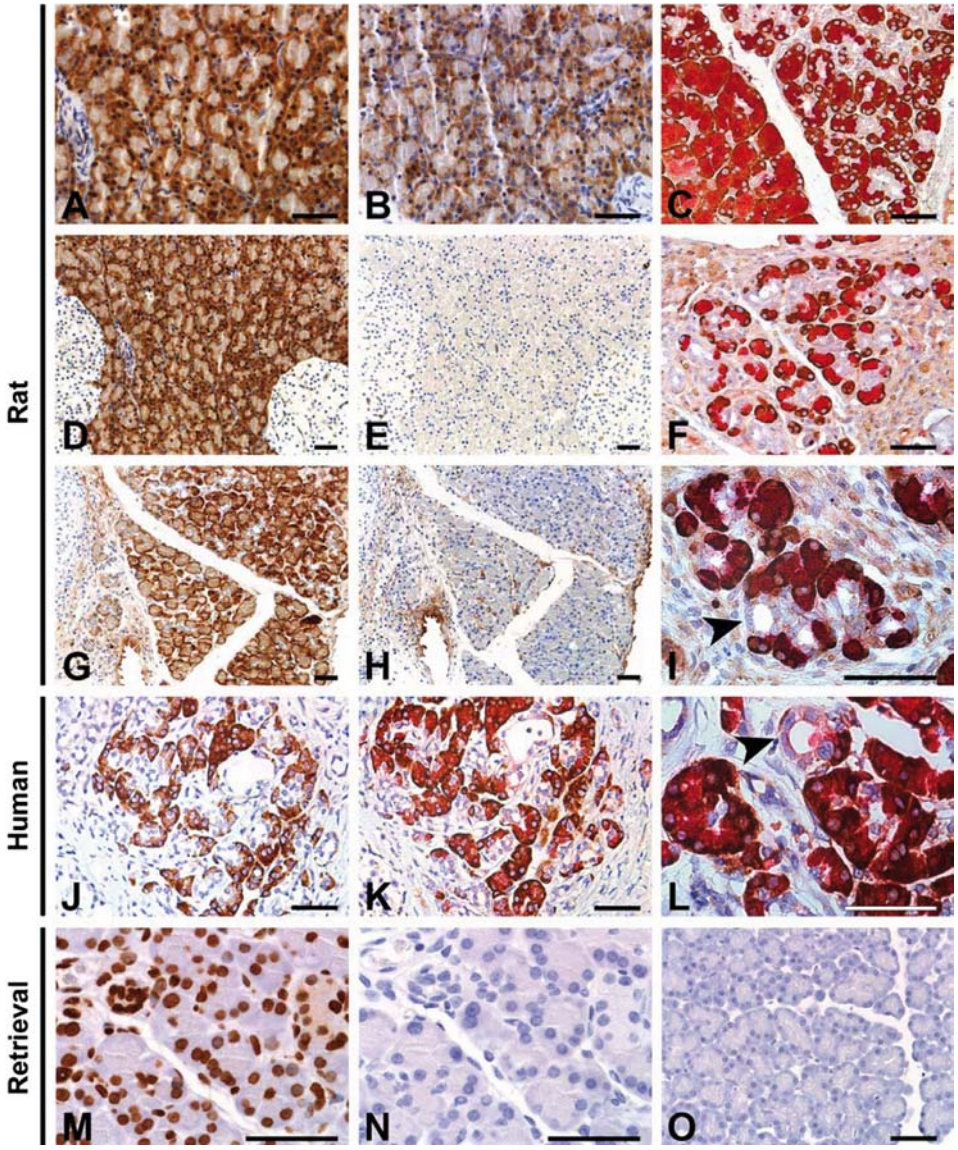

Figure 1: Immunohistochemistry of SPARC (brown) and pancreatic stone protein PSP (red) in tissue sections from rat and human pancreatic specimen. Proteinase K treatment retrieves cytoplasmic SPARC. A-I and M-O rat pancreas, J-L human pancreas; A,D,G identify SPARC in WBN/Kob rats, B in Wistar rat, J in human. E, H adjacent control section (no 1° antibody) for D and G.C, F, I (rat), K and L (human) show double staining of SPARC and PSP. M and N: Microwave pre-treatment shows staining of SPARC in the nuclei (M) while in the respective controls (N) no staining is visible. Without pre-treatment (O), SPARC could not be detected. Arrows point to tubular structures. Bars equal 50µm.

Role of serotonin in disease and regeneration of the pancreas

Dr. A. Silva, M. Bain, Dr. W. Moritz, PD Dr. R. Graf

For nearly all of the patients suffering from chronic pancreatitis, the condition is associated with debilitating pain and progressive pancreatic dysfunction which results in deficient digestion, the onset of diabetes and in many cases pancreatic cancer. Despite the incidence of chronic pancreatitis in industrialised countries (about 3.5-10 per 100,000 inhabitants), therapy and treatment is limited to pain and diabetes management, as well as enzyme therapy. For pancreatic cancer patients, the outcome is even worse as often they require nearly full pancreatectomy.

In mammals, the liver is known to inherit a strong regenerative potential. A number of reports have also shown the endocrine pancreas to be able to regenerate following insults such as physical ligation of the pancreatic duct, chemical toxins or virus-induced diabetes. Furthermore, the process of pancreatic regeneration can also be preceded by an operative insult that involves removal of part of the organ, and has been demonstrated in animal models and in humans. Indeed, rat models show a rapid increase in labelling indices in centroacinar cells as well as cells of the ductal system as early as 2 days after 90% pancreatectomy, including regenerative stem cells for insulin producing β cells. These studies were further validated by the observation of parental cells of regeneration present in human ductal epithelium.

The focus of pancreatic regeneration has been centered in the endocrine pancreas, namely in pancreatic islets and β-cell regeneration. However, it is equally important to understand the growth and regenerative potential of the exocrine pancreas in patients that have chronic pancreatitis and/or pancreatic cancer. The regeneration and maintenance of pancreatic endocrine and exocrine function is likely to have a significant therapeutic impact in patients affected by pancreatic diseases. In pancreatectomy models, there are a number of growth factors and gut hormones which appear to be promising candidates. Nevertheless, little is known about the precise role of these components which drive regeneration in the pancreas. A great deal more is known about the liver, and due to their close developmental and functional activities, one can predict common mechanisms of regeneration. Indeed, the liver and pancreatic lineages arise from a common embryonic endoderm cell population in the anterior foregut. Decision between a hepatic or pancreatic fate is thought to be regulated by only few key developmental steps, such as Fibroblast Growth Factor (FGF) from the adjacent heart promoting the hepatic over a pancreatic fate, and the subsequent expression of Pdx-1 in the presumptive pancreatic lineage. The main cell type in the liver – hepatocytes – and in the pancreas – acinar cells – share many common features. In addition, both organs exhibit quiescent resident stellate cells which upon activation switch their phenotype and start expressing α-Smooth Muscle Actin [α-SMA], are rich in vitamin A and express desmin.

Serotonin (5-hydroxytryptamine; 5-HT), a potent vasoconstrictor which is mainly carried by platelets, appears to be involved in the proliferation of fibroblasts, epithelial cells, macrophages and other immune/inflammatory cells. Furthermore, it has been shown that in the liver, serotonin is crucial to liver regeneration. In mice where tryptophane hydroxylase 1(TPH 1), a critical enzyme in the cascade synthesis of serotonin - is knocked out, liver regeneration is greatly reduced following hepatectomy. In the current project, we have focused on proteins responsible for serotonin production and signalling. After chronic inflammatory insults, these proteins appear to be upregulated (Figure 2). Furthermore, mice deficient in peripheral serotonin (TPH 1 -/-) exhibit an increased inflammatory response (Figure 3).

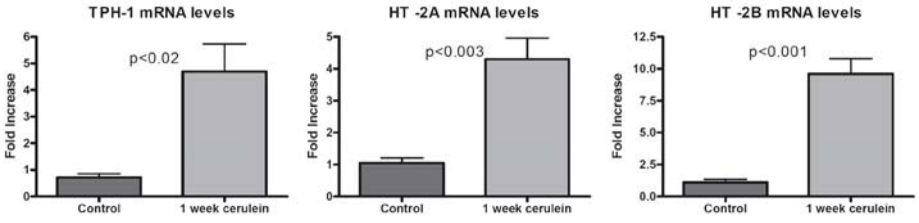

Figure 2: Serotonin dependent TPH-1 enzyme and serotonin receptors are up-regulated after cerulein treatment. As shown, wild type B6 animals have increased pancreatic mRNA levels of TPH-1 and serotonin receptors HT -2A and HT -2B following cerulein treatment when detected in the pancreas by real time PCR. Similar results were found following 60% pancreatectomy.

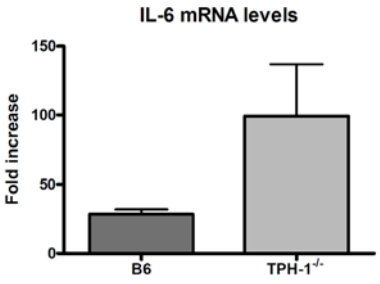

Figure 3: Pro-inflammatory genes are up-regulated in serotonin deficient mice. As shown, TPH-1-/- animals have increased levels of pro-inflammatory genes compared to wild type mice (B6)

Interaction of PSP/reg and PAP with stellate cells
PD Dr. R. Graf

Pancreatic stellate cells (PSC) play a central role in fibrogenesis associated with acute and chronic pancreatitis. Pancreatic stone protein/regenerating protein (PSP/reg) belongs to a family of secretory stress proteins (SSP) which is constitutively synthesized by pancreatic acinar cells and upregulated dramatically during acute and chronic pancreatitis. Recently we have shown interactions of pancreatitis-associated protein, another member of the SSP-family, with stellate cells.

Assuming a protective role of PSP/reg we investigated its effects on human PSC. PSC were obtained by outgrowth from fibrotic human pancreas tissue. Recombinant PSP/reg was added to cultured PSC.

Cell proliferation was determined by bromodeoxyuridine incorporation. PSC migration was assessed by a wound healing assay. Extracellular matrix (collagen type I and fibronectin), matrix metalloproteinases (MMPs), tissue inhibitors of matrix metalloproteinases (TIMPs) and reversion- inducing cysteine-rich protein with Kazal motifs (RECK) were demonstrated on protein and mRNA level.

PSP/reg inhibited PSC proliferation and migration in a dose-dependent manner. Soluble collagen I and fibronectin were reduced after addition of PSP/reg. PSP/reg had no effect on MMP-1 (matrix metalloproteinase-1), slightly decreased the synthesis of MMP-2 and strongly decreased tissue inhibitors of MMP-1 (matrix metalloproteinase -1) and -2 concentrations in PSC supernatants. Cysteine-rich protein with Kazal motifs (RECK) expression was induced after PSP/reg application (Figure 4). Our data provide evidence that in vitro, PSP/reg reduces fibrogenesis and stimulates fibrolysis. The findings suggest that PSP/reg may have a protective function in the repair phase of acute and chronic pancreatitis by promoting resolution of fibrosis. We highlight PSP/reg as an antifibrogenic protein in pancreatic injury.

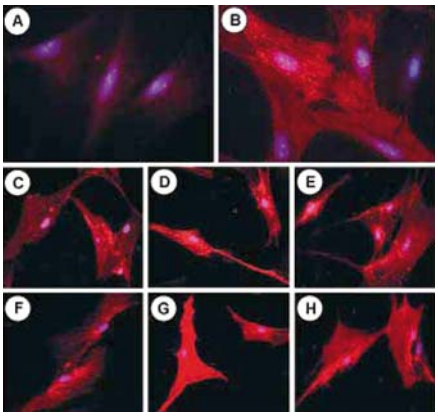

Figure 4. Fluorescence micrographs showing the immunoreactivity of reversion-inducing cysteine-rich protein with Kazal motifs (RECK) on the cell surface of cultured human PSC. Cultured human PSC were incubated with recombinant PSP/reg. After 24 and 48 hours, cultures were fixed and Alexa Fluor 568 immuno-stainings were performed. (A) Without first antibody, all three cells are negative. Nuclear counterstaining was performed with bisbenzimide. (B) Positive RECK staining in human PSC. The amount of RECK expression on the cell surface is different from cell to cell, and also few negative cells were observed. (C-E) Fixation 24 hours; (F-H) Fixation 48 hours; (C and F) Control without PSP/reg; original magnification, 100×.

Achievements 2008

- Dr. A. Silva received a poster prize at the ‚Day of Clinical Research'
- Dipl. phil. Th. Reding received the basic science poster prize at the annual meeting of the European Pancreatic Club in Poland.

Collaborations:

- Dr. Mathias Heikenwälder, Institut für Neuropathologie, Universitätsspital Zürich
- Dr. Achim Weber, Institut für klinische Pathologie, Universitätsspital Zürich
- Dr. Pia März & Prof. U. Otten, Physiologisches Institut, Universität Basel
- PD Dr. Marius Keel & Dr. Luc Härter, Klinik für Unfallchirurgie, Universitätsspital Zürich
- Dr. Martin Hersberger, Institut für Klinische Chemie, Universitätsspital Zürich
- Prof. M. Bachem, Klinische Chemie, Universitätsklinikum, Ulm

Selected references:

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- Ling Li , Max G. Bachem, Shaoxia Zhou, Zilin Sun, Jinfei Chen, Marco Siech, Daniel Bimmler, Rolf Graf. Pancreatitis-Associated Protein Inhibits HumanPancreatic Stellate Cell MMP-1 and -2, TIMP-1 and -2 Secretion and RECK Expression. Pancreatology 2009;9:99–110

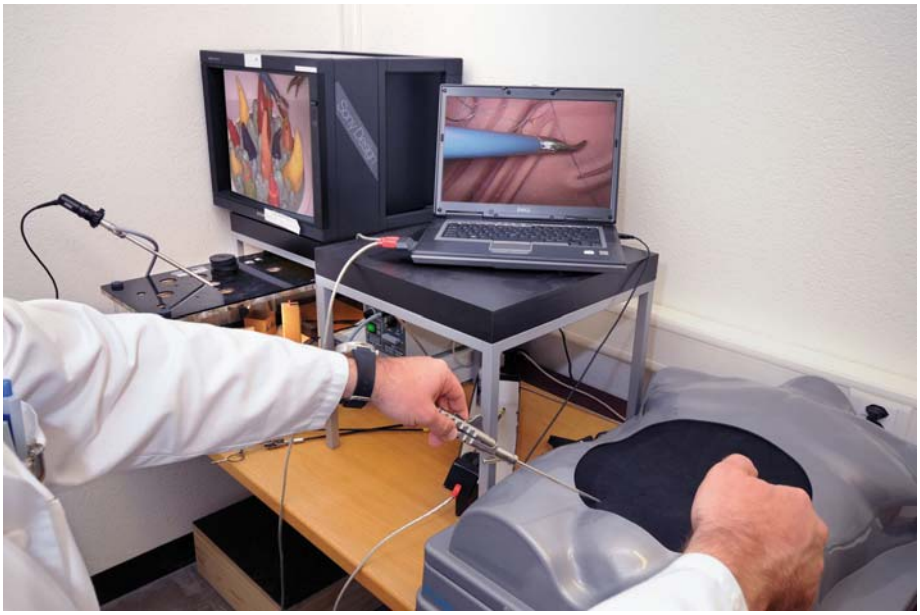
2.2.3 Surgical Skill Center

Clin. Ass. Prof.
PD Dr. med.
Dieter Hahnloser

Jérôme Gapany

PD Dr. med. Dieter Hahnloser, Jérôme Gapany

The use of virtual reality (VR) has gained increasing interest to acquire laparoscopic skills outside the operating theatre and thus increasing patients‘ safety. We valuated 735 surgical trainees from 28 countries at the International Gastrointestinal Surgery Workshop 2006-2008, held in Davos, Switzerland. The possibility of using VR at the courses was estimated as excellent or good in 68%and useful in 21%. If such VR simulators were available at their institution, most course participants would train at least one hour per week (46%), two or more hours (42%) and only 12% wouldn‘t use VR. Similarly, 63% of the participants would accept to operate on patients only after VR training and 55% to have VR as part of their assessment. We could demonstrate that residents accept and appreciate VR simulation for surgical assessment and training. The majority of the trainees are motivated to regularly spend time for VR training if accessible (Patient Saf Surg. 2008 Jun 11;2:16).
In 2008 we started with several studies providing data for a for proficiency-based VR training program. These studies are part of a close collaboration of the University of Lausanne and Zürich supported by a SNF grant. We further founded the Swiss Study Group for Surgical Simulations (www.swiss-sim-surg.ch) providing a platform for continuous skills training for all residents.



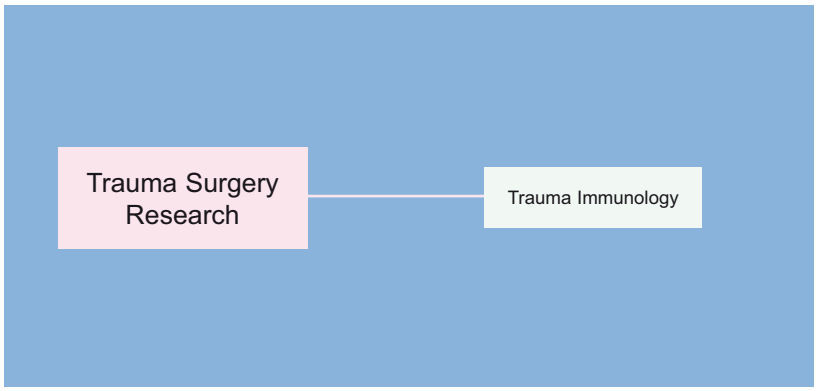
2.3 Trauma Surgery Research


PD Dr. med.
Marius Keel


Prof. Dr. med.
Otmar Trentz


PD Dr. med.
Guido Wanner


Prof. Dr. med.
Hans-Peter Simmen



2.3.1 Trauma Immunology


PD Dr. med.
Marius Keel


Dr. rer. nat.
Luc Härter


Dr. med.
Emanuel Benninger


Dr. med.
Philipp Lenzlinger


Dr. med.
Ladislav Mica


Dr. med.
Thomas Lustenberger


Dr. med.
Mario Rancan


Ursula
Steckholzer

Pancreatic stone protein (PSP) is highly increased during post-traumatic sepsis and activates neutrophil granulocytes
PD Dr. med. Marius Keel MD, Dr. rer. nat. Luc Härter

The post-traumatic course after severe injury can be complicated by sepsis and/or multiple organ failure, conditions with a high mortality. Among the most commonly used markers of systemic infection and sepsis are leukocyte counts, C-reactive protein (CRP) and procalcitonin (PCT). The latter are two serum proteins highly induced after trauma, yet without any known function. So far, reliable predictors and indicators of post-traumatic sepsis are not available and hence treatment may lag behind the onset of sepsis.
Pancreatic stone protein/regenerating protein (PSP/reg), a secretory protein produced in the pancreas, dramatically increases during pancreatic disease. Based on experiments in animals in which PSP/reg was increased after stress we hypothesized that this protein might also be increased in patients after a non-pancreatic trauma. To investigate whether subgroups, based on clinical criteria of infection and sepsis, would respond differentially we determined post-traumatic serum PSP/reg levels.
PSP/reg serum levels from 83 patients with polytrauma (ISS ≥ 17 points) but without pancreatic damage were compared to serum from healthy controls (n=38). PSP/reg serum levels were related to known inflammation markers such as c-reactive protein, IL-6, procalcitonin and leukocyte numbers.
Expression of CD62L and CD11b on neutrophils was measured after staining with fluorescence-labeled antibodies in cytometer (FACS) as well as binding of FITC-labeled PSP/reg. 33 patients (39%) developed sepsis, 32 (38%) had local infections and 18 (21%) were free of infections, 11 (13%) patients died. Initial (Day 0) serum levels of PSP/reg in all three patient groups (10.5 [7.4-15.2]; 10.9 [5.1-14.8]; 10.6 [6.9-16.3] ng/mL, median [interquartile range]) were comparable to healthy controls (n=38; 10.4 [7.5-12.3] ng/mL). After day 3 serum levels were significantly elevated in patients with sepsis (146.4ng/mL), and after day 5 in patients with infections (111.4ng/mL) compared to

patients without infections (22.8ng/mL) (Figure 1). The courses of PSP/reg in the three groups were significantly different (all p < 0.0005 after Bonferroni correction). No Bonferroni correction was performed for comparisons between groups at single days. Furthermore, we could demonstrate binding of FITC-labeled recombinant PSP/reg to human neutrophils. A dose-dependent increase of FITC-fluorescence (from 7,7 ± 0,8 to 28,2 ± 0,8 MFI) was detected on PMN, whereas autofluorescence was low (4,6 ± 0,2 MFI). After co-incubation with 100-fold non-labeled excess of PSP/reg (n=3), the FITC-PSP/reg fluorescence in PMN (MFI 28,2 ± 0,8) was significantly reduced (8,9 ± 2,4 MFI), demonstrating specificity of PSP/reg binding to PMN.

Recombinant PSP/reg elicited a dose-dependent shedding of L-selectin (CD62L) and up-regulation of β2-integrin (CD11b) in neutrophils indicating that PSP activates neutrophils. Incubation of PMN (10^6/mL) for 1h with PSP/reg (500ng/mL) induced a significant reduction of CD62L fluorescence in PMN from healthy controls, indicating shedding of CD62L. In contrast, CD11b was significantly up-regulated in PMN stimulated with PSP/reg.

We conclude that PSP/reg is up-regulated in blood after trauma and correlates with severity of inflammation. Furthermore, PSP/reg binds to and activates neutrophils. Therefore, PSP/reg might be an acute phase protein and may serve as a marker for post-traumatic complications.

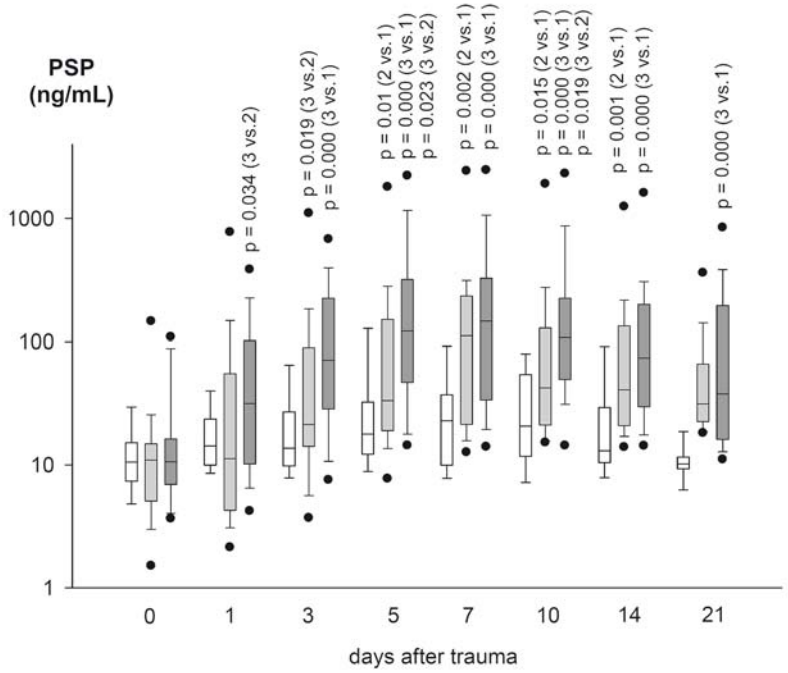


Fig. 1. Kinetics of PSP/reg serum levels in polytrauma patients. PSP/reg serum levels from polytrauma patients without infection (1, white bars, n= 18), with local infection (2, grey bars, n= 32) or sepsis (3, darkgrey bars, n= 33) were measured by ELISA from day of admission until day 21. Data is given as box-and-whiskers plots with a median and 5^{th}/95^{th} percentiles. Exact P values for different comparisons are depicted, p=0.000 indicate P values smaller than 0.0005.

Patentanmeldung: „Method for Assaying Sepsis in Humans„ Europäische Prioritätsanmeldung, Filing Number: EP 07/017539.3 Filing Date: 07.09.2007

Achievements 2008

- Participation in the worldwide largest trauma F7 Trauma-1711 phase III study: “A multi-center, randomized, double-blind, parallel group, placebo controlled trial to evaluate the efficacy and safety of activated recombinant factor VII (rFVIIa/NovoSeven®/NiaStase®) in severely injured trauma patients with bleeding refractory to standard treatment”. The Division of Trauma Surgery, University Hospital Zürich with Dr. M. Keel as the principle investigator (PI) recruited a total of 22 patients. The study was terminated due to a negative futility analysis result by the sponsor.

Collaborations:

- PD Dr. R. Graf, Division of Visceral & Transplant Surgery, USZ, Zürich
- PD Dr. J. Stover, Clinic for Intensive Care Medicine, USZ, Zürich
- PD Dr. M. Zaugg, Clinic for Anesthesiology, USZ, Zürich
- Prof. Dr. D. Demetriades, Director of Trauma / Surgical Critical Care, University of Southern California, Los Angeles, USA

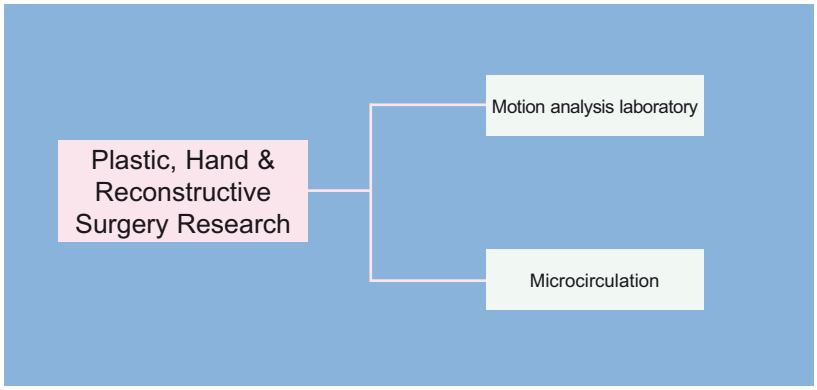
Selected references:

- In vitro norepinephrine significantly activates isolated platelets from healthy volunteers and critically ill patients following severe traumatic brain injury. Tschuor C, Asmis LM, Lenzlinger PM, Tanner M, Härter L, Keel M, Stocker R, Stover JF. Crit Care. 2008;12(3):R80.
- Delayed inhibition of agonist-induced granulocyte-platelet aggregation after low-dose sevoflurane inhalation in humans. Wacker J, Lucchinetti E, Jamnicki M, Aguirre J, Härter L, Keel M, Zaugg M. Anesth Analg. 2008 Jun;106(6):1749-1758.
- Measures of endothelial dysfunction in plasma of patients with posttraumatic stress disorder. von Känel R, Hepp U, Traber R, Kraemer B, Mica L, Keel M, Mausbach BT, Schnyder U. Psychiatry Res. 2008 Apr 15; 158(3):363-373.
- Operative repair or endovascular stent graft in blunt traumatic thoracic aortic injuries: results of an American Association for the Surgery of Trauma Multicenter Study. Demetriades D, Velmahos GC, Scalea TM, Jurkovich GJ, Karmy-Jones R, Teixeira PG, Hemmila MR, O‘Connor JV, McKenney MO, Moore FO, London J, Singh MJ, Lineen E, Spaniolas K, Keel M, Sugrue M, Wahl WL, Hill J, Wall MJ, Moore EE, Margulies D, Malka V, Chan LS; American Association for the Surgery of Trauma Thoracic Aortic Injury Study Group. J Trauma. 2008 Mar;64(3):561-570; discussion 570-571.
- Reduced midazolam clearance must be considered in prolonged coma. Meierhans R, Stover JF, Béchir M, Keel M, Stocker R. Anaesth Intensive Care. 2008 Nov;36(6):915-916.
- Differential temporal profile of lowered blood glucose levels (3.5 to 6.5 mmol/l versus 5 to 8 mmol/l) in patients with severe traumatic brain injury. Meier R, Béchir M, Ludwig S, Sommerfeld J, Keel M, Steiger P, Stocker R, Stover JF. Crit Care. 2008;12(4):R98.

2.4 Plastic, Hand & Reconstructive Surgery Research

Dr. med.
Maurizio Calcagni

Prof. Dr. med.
Pietro Giovanoli

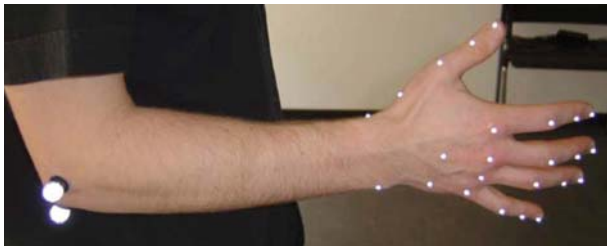


2.4.1 Motion Analysis Laboratory

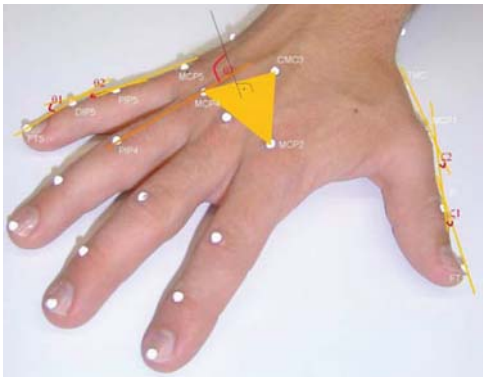
Dr. med.
Maurizio Calcagni

In vivo assessment of wrist and small finger joints motion
Dr. Maurizio Calcagni, Alexa Stähli, Dr. H. Gerber, Prof. Dr. E. Stüssi

In 2008 a Master Thesis was produced. The marker set for the measurements in vivo assessment of wrist and small finger joints is now established. We use a set of markers which allows us to analyze motion 3-D during flexion, extension, pronation and supination.



Skin markers of the kinematic model of wrist and small finger joints



Geraden und Ebenen zur Berechnung der Winkel bei der Flexion/Extension der Finger und des Daumens

A major part of this work was the development of a Matlab program for the calculation of angles and angular velocity. Twenty healthy volunteers were measured during standard joint motion of fingers and wrist. Our measurements are in the reported range of the literature. This work is the basis for the development of the tools for clinical application.

Achievements 2008

Talks

- Invited speaker at the XIVth FESSH Congress.
  Instructional Course Osteosynthesis in the Hand, Lausanne 2008
  Examiner of the European Board of Hand Surgery
  Scientific Director 4th Zurich Workshop on Handflaps. University of Zurich

Collaborations:

- Departement of Motion Analysis Science of the ETH Zurich
  (Dr. Hans Gerber, Prof. Dr. E. Stüssi)
  Master in Motion Analysis Science: Alexa Stähli “Development of a set of markers for the hand and the wrist”
- Departement of Drug Delivery Systems of the ETH Zurich (Prof. Gander)
  Master in Pharmacology: Srinivas Madduri „Peripheral nerve regeneration in rats through nerve conduits releasing neurotropic factors“

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- M. Calcagni, I. Tami, P. Giovanoli: Osteosynthesis of the upper extremity in presence of infection: diagnosis and treatment rationale.
  In Osteosynthesis in the Hand: current concepts, Ed. D.B. Herren, L. Nagy, D.A. Campbell, 2008.

2.4.2 Microcirculation

Dr. med.
Claudio Contaldo

Dr. med.
Nicole Lindenblatt

Dr. med.
Uwe Platz

Dr. med.
Sebastian Vetter

Dr. med.
Alexander Handschin

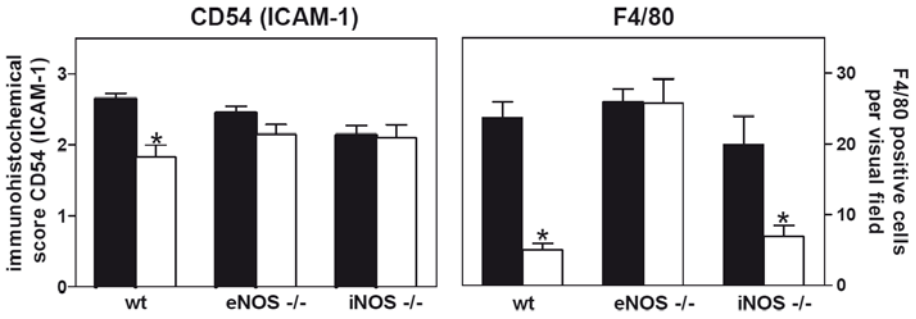
Chen Fanfan
PhD Student

cand. med.
Dominik Högger

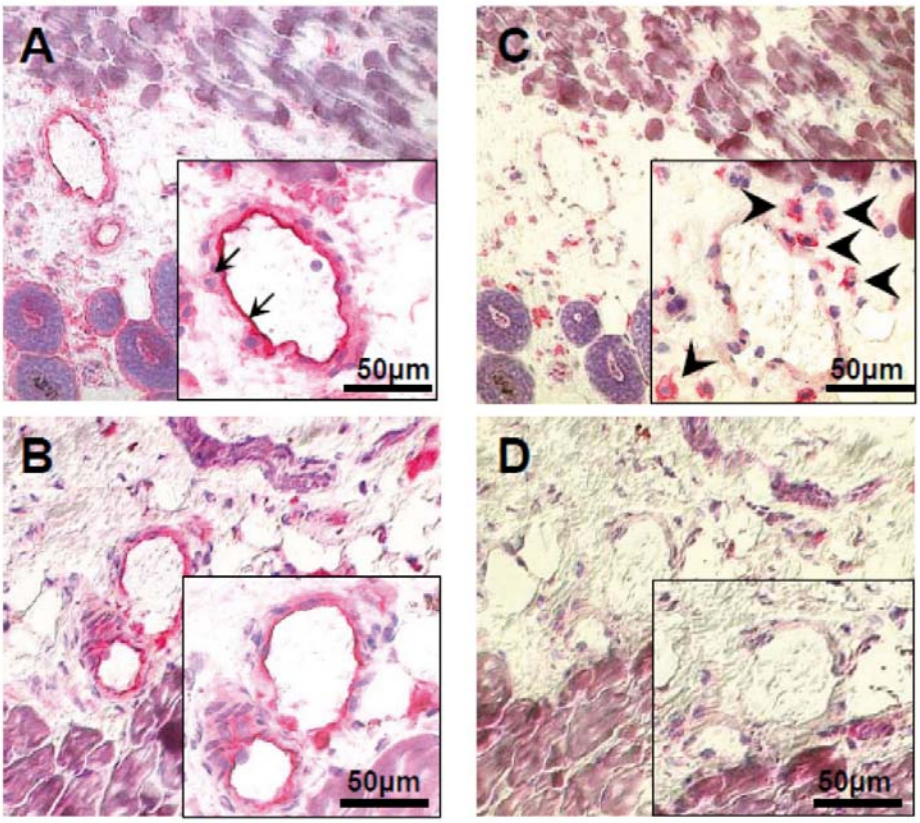
Erythropoietin reduces TNF-alpha induced inflammation via eNOS-dependent deactivation of F4/80 cells

Dr. med. Claudio Contaldo, Dr. med. Nicole Lindenblatt, Dr. med. A. Elsherbiny, Dr. med. Karl Lang, Dr. med. Sebastian Vetter, Dr. med. Alexander Handschin, Dr. med. Maurizio Calcagni, Prof. Dr. med. Pietro Giovanoli

Erythropoietin (EPO) lately has been shown to protect cells against damage caused by diverse pathological conditions, such as ischemia-reperfusion, trauma and inflammation. The increase of nitric oxide (NO) production is advocated as one major mechanism to explain these beneficial properties of EPO. To gain further insight in the underlying mechanisms of the effects of EPO in the setting of TNF-alpha-induced inflammation, we studied microcirculatory leukocyte and F4/80 positive cell behaviour, nutritive tissue perfusion and apoptosis in an in vivo mouse model. Since NO production is suspected to be a key factor we evaluated the effects of EPO application on these parameters in wild type and NO-deficient mice. TNF-alpha application induced leukocyte sticking and rolling, as well as apoptosis and decreased nutritive tissue perfusion. EPO was able to significantly improve all these parameters in wild type-mice. In contrast to this these protective properties of EPO were not observed in eNOS knock out mice, suggesting endothelial derived NO as main anti-inflammatory factor. Moreover, F4/80 positive cell invasion into the perivascular tissue was likewise significantly diminished proposing mast cell deactivation as potentially important factor in EPO-mediated tissue protection. However, the anti-apoptotic effect of EPO was maintained in both iNOS and eNOS knock out mice, indicating that these mechanisms might rather be conveyed by other factors. Concludingly it can be stated that we revealed a new mechanism of EPO in the suppression of inflammatory damages within the microcirculation – the dampening of NO-regulated F4/80 cell infiltration into the tissue. This entails a reduced intravascular leukocyte activation and subsequently extravasation. The main mechanism of EPO-mediated mast cell deactivation appears to be the presence and up-regulation of eNOS within the vessel wall.



Immunohistochemical expression of CD 54 in vascular endothelial cells and perivascular staining with F4/80 antibody that detects monocytes/ macrophages in longitudinal sections of the mouse dorsal skinfold 30min after exposure to TNF-alpha in vehicle-treated (solid bars) and EPO-treated (open bars) wild type (WT)-, eNOS depleted- (eNOS-/-) and iNOS depleted mice (iNOS-/-). Staining intensity of ICAM-1 was determined by a semiquantitative score (0 = no, 1 = weak, 2 = moderate, 3 = strong staining). Values are means ± SD (n = 5 per group).
Data are mean ± SD. *P < 0.05 vs vehicle.



Immunohistological demonstration of CD 54 (A and B) expression (red staining) of endothelial cells (arrows) and F4/80 (C and D) expression (arrowheads) that detects monocytes/ macrophages in cross sections of mouse striated muscle venules 30min after exposure to TNF-alpha in vehicle-treated (A and C) and EPO-treated (B and D) wild type animals. Note strong expression of CD 54 in endothelial cells upon TNF-alpha exposure and almost complete prevention of perivascular accumulation of macrophages/ monocytes after EPO pretreatment 1h before inflammatory stimulation.

Revascularization of skin grafts in a new in vivo model

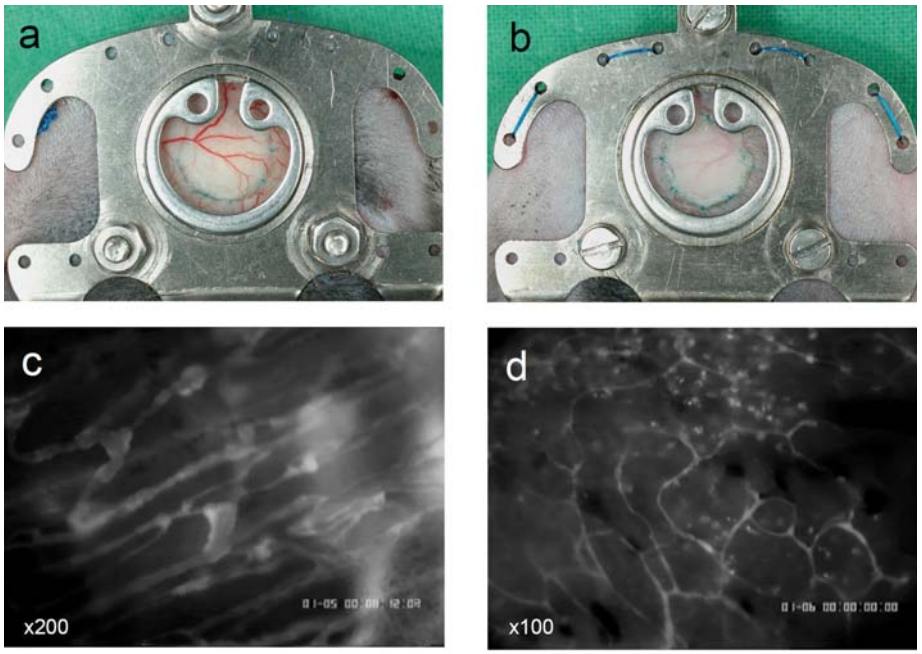
Dr. med. Nicole Lindenblatt, Dr. med. Claudio Contaldo, Dr. med. Maurizio Calcagni, Uwe Platz, Prof. Dr. med. Pietro Giovanoli

Skin grafting still represents the “gold standard” to cover skin defects. Despite widespread clinical use the exact process of skin graft revascularisation remains unclear. Next to this the development of skin substitutes and their repeated failure to acquire adequate blood supply, demands renewed efforts to understand the vascularization of skin grafts. Therefore we developed a new animal model allowing for continuous monitoring of the microcirculation during revascularization of a skin graft. The aim is, to be able to visualize the microvascular architecture during engraftment, and, by this, to gain further insights into the time course and potential vascular transformations during revascularization.

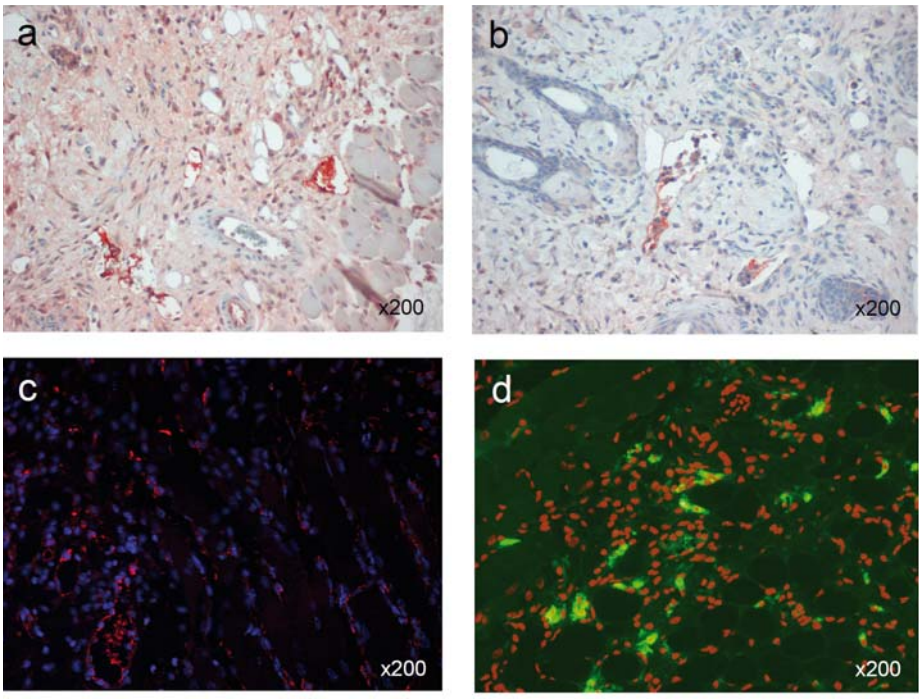
The preparation of the modified dorsal skinfold chamber enabled us to simultaneously study the microcirculation of both the wound bed and the skin graft by intravital microscopy over a time period of 10 days. In addition, immunohistochemistry of angiogenic factors and corrosion casting were performed to further characterize the process of revascularization.

Capillary buds and sprouts firstly appeared at 48 hours. Initial filling of graft capillaries occurred at 72 hours, increasing over the following days and resulting in almost complete restoration of the original pattern of skin microcirculation after 120 hours. After 96 hours bud-like structures could be detected at the capillary divisions of the grafts, most probably reflecting an angiogenic response to factors secreted by the ingrowing vessels. Immunohistochemical analysis exhibited strong and selective staining for HIF-1α within the grafts and of VEGF, Ang-1 and Flt-1 within the wound bed. Corrosion casting showed the formation of new capillaries, originating from the wound bed and connecting to the graft vasculature.

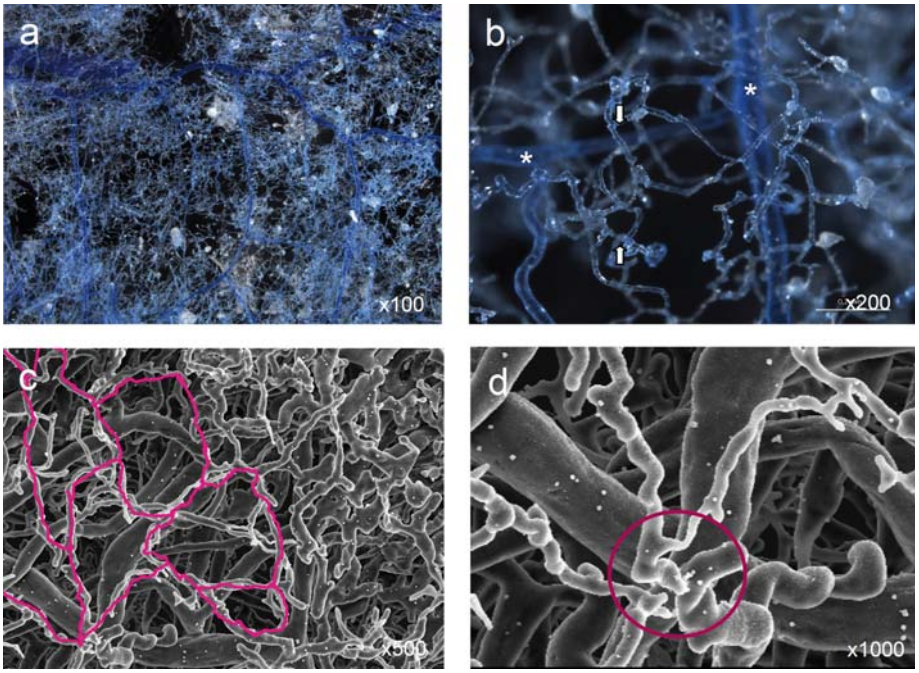
These data indicate an early onset of angiogenesis, resulting in complete revascularisation of the skin graft at day 5. The fact that the graft retained its original capillary pattern indicates an inosculation of the original graft vasculature with the newly formed blood vessels of the wound bed. Most likely, hypoxia within the graft caused an up-regulation of HIF-1a, which then triggered the increased VEGF expression within the wound bed, leading to the induction of angiogenic factors and receptors, and, eventually, angiogenesis. Vessels connecting to the graft vasculature most likely cause a temporary immature vessels structure or antransient angiogenic response within the graft.



Preparation of the modified dorsal skinfold chamber: From the front of the chamber (a) the muscular wound bed (panniculus carnosus) and the larger subcutaneuous vessels are visible and accessible to intravital microscopy. Simultaneously the microcirculation of the
skin graft can be visualized in vivo in the back (b). The wound bed showed a strong angiogenic response after 48 h (c) leading to reperfusion of the graft capillaries after 72 h (d).



The wound bed exhibited the expression of the hypoxia inducible factor HIF-1α (a)
and subsequently VEGF (b), Ang-1 (c) and the VEGF-receptor Flt-1 (d). This indicates that angiogenesis in the context of skin graft revascularisation most likely is hypoxia-driven.



Corrosion casting and subsequent light micrsocopy revealed a dense tridimensional microvascular network of newly formed vessels within the wound bed after skin grafting (a). At a higher magnification the vessels of the wound bed (asterisks) as well the loop-like oriented vessels of the graft (arrows) can be identified (b). SEM confirms angiogenesis within the wound bed and reperfusion according to the original pattern of graft vascularisation (c). At high magnifications connections between newly developed tortous vessels from the wound bed to the graft capillaries can be identified (d).

Effects of Extracorporeal Shock Wave Energy on Normal Murine Microcirculation

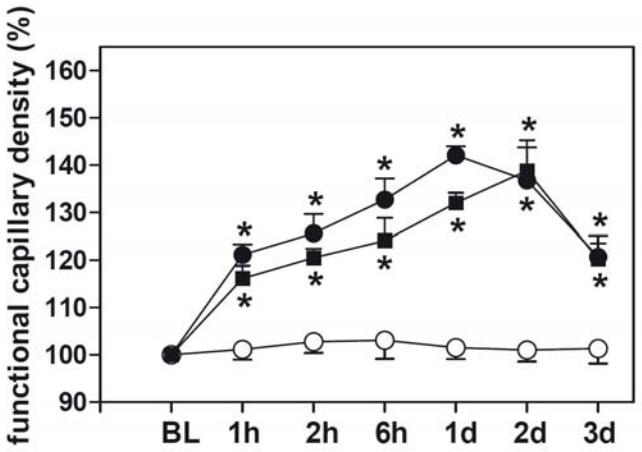
Dr. med. Claudio Contaldo, Dr. Fanfan Chen, cand med. Dominik Högger, Dr. med. Natascha Forster, Dr. med. Nicole Lindenblatt, Dr. med. Sebastian Vetter, Dr. med. Maurizio Calcagni, Prof. Dr. med. Pietro Giovanoli

Shock wave energy generated for medical purposes is characterized by a biphasic pressure pulse, whereby a cavitation phenomen is induced causing the formation of tiny vesicles. As these microvesicles collapse a multidirectional fluid stream is induced within the tissue. Recently the application spectrum of extracorporeal shock waves (esw) has been widened from treatment of urinary calculi and bony non-union to soft tissue pathologies such as chronic wounds and flap ischemia. The aim of this study was to assess the effects of esw energy on normal microcirculation.

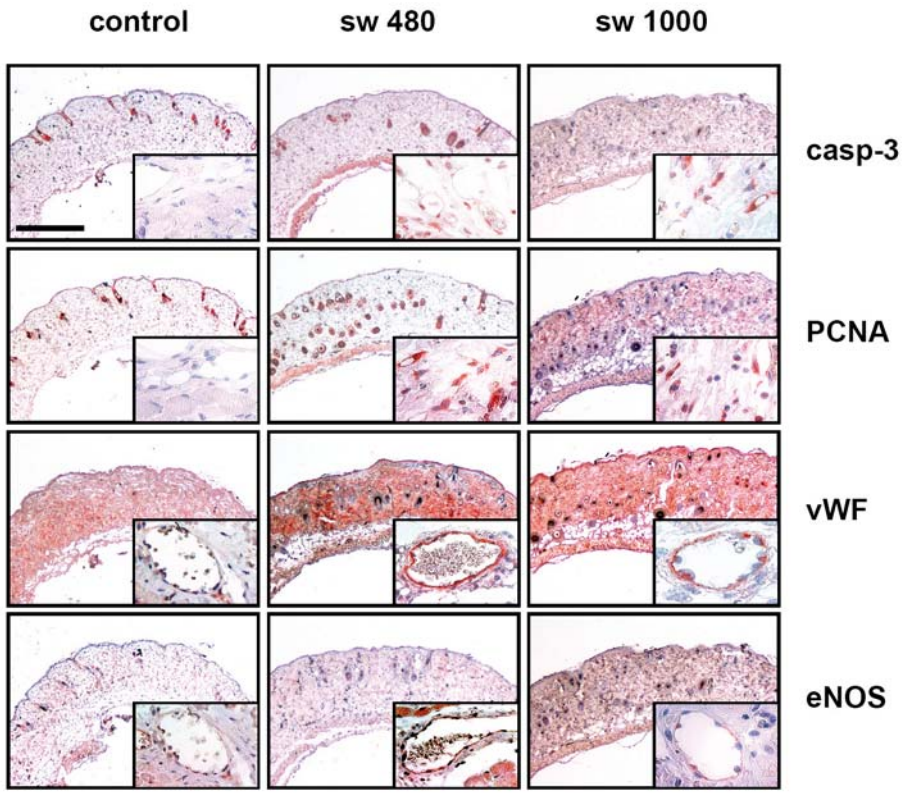
The mouse dorsal skinfold chamber model was used to study quantitatively the microhemodynamics by intravital fluorescence microscopy 1h, 2h, 6h and 24h after esw impact. The study comprised one treatment group (n=5) and one untreated control group (n=5). Animals were once treated with a dose of 500 impulses at an energy flux rate of 0.08 mJ/mm2. Quantitative analysis of microvascular perfusion and leukocyte-endothelial cell interaction was assessed after contrast enhancement with 5% FITC-dextran. We used topical bisbenzimyde and immunohistochemical staining of caspase-3 to assess tissue apoptosis.

Esw energy caused a significant increase of functional capillary density (fcd) after1h to 125% ± 2, after 2h to 129% ± 5, reaching a maximum after 24h to 140% ± 2 of baseline ($p<0.01$, all time points). We observed a slight increase (~2-fold, $p<0.05$) of rolling leukocytes in postcapillary venules. We didn't find any significant changes in arteriolar, venular and capillary diameters and red blood cell velocities. In line with this, arteriolar and venular wall shear rates didn't show any changes. In vivo analysis of nuclear condensation showed a 2.6 fold ($p<0.05$) increase of apoptotic cell count which was verified by an increased expression of caspase-3.

Our data suggests that esw application induces a slight and transient inflammatory reaction. Of interest we observed a significant increase of capillary perfusion which may be caused by the recruitment of nonperfused capillaries and might have therapeutic significance in future.



Functional capillary densities (given in percent changes of baseline) in the mouse dorsal skinfold chamber of sham-treated animals (○), and after extracorporeal shockwave application (ESWA) immediately after baseline (BL) with 500 pulses (■), or 1000 pulses (●) at an energy flux rate of 0.08 mJ/mm2 and a frequency of 8 Hz. Analyses were performed at baseline (BL) as well as at 1h, 2h, 6h, and 1d, 2d and 3d after ESWA. Note the Data represent mean values ± SD. *P < 0.05 vs. BL and sham-treated animals at corresponding time points; #P < 0.05 vs. 500 pulses at corresponding time point.



Immunohistological demonstration (red staining) of caspase-3, proliferating cell nuclear antibody (PCNA), endothelial nitric oxide synthase (eNOS) and von Willebrand factor (vWF) in cross sections of the mouse dorsal skinfold 1d after extracorporeal shockwave application (ESWA) with 500 pulses or 1000 pulses at an energy flux rate of 0.08 mJ/mm2 and a frequency of 8 Hz compared to normal expression in sham-operated animals. Note strong expression of PCNA in the interstitium (arrowheads) and vWF as well as eNOS in endothelial cells (arrows) following ESWA. Note also a marked accumulation of caspase-3 positive apoptotic cells in the tissue after 1000 pulses.

Achievements 2008

Talks

- C. Contaldo, A. Elsherbiny, D. Högger, S. Vetter, N. Lindenblatt, M. Calcagni, P. Giovanoli. Erythropoietin improves wound healing by increasing red blood cell perfusion in hypercholesteremic mice. European Congress of Scientists and Plastic Surgeons (ECSAPS). Bern, 12.09.2008.
- C. Contaldo, C. Fanfan, N. Forster, S. Vetter, N. Lindenblatt, M. Calcagni, P. Giovanoli. Effects of Extracorporeal Shock Wave Energy on Normal Murine Microcirculation. European Congress of Scientists and Plastic Surgeons (ECSAPS). Bern, 13.09.2008.
- C. Contaldo, A. Elsherbiny, D. Högger, S. Vetter, N. Lindenblatt, M. Calcagni, P. Giovanoli.Erythropoietin improves wound healing by increasing red blood cell perfusion in hypercholesteremic mice.
  44th Annual Meeting of the Swiss Society of Plastic, Reconstructive and Esthetic Surgery. (SGPRAEC), Lausanne 04.10.08
- Dr. Claudio Contaldo was invited as a chairperson in the Microcirculation session of European Congress of Scientists and Plastic Surgeons (ECSAPS). Bern, 13.09.2008.
- Lindenblatt N, Menger MD, Giovanoli P, Vollmar B. Characterisation of the revascularisation of skin grafts in a new in vivo model– the role of angiogenesis. Annual Congress of the European Association of Plastic Surgeons (EURAPS). Madeira, 30.05.2008.
- Lindenblatt N, Calcagni M, Schmidt CA, Contaldo C, Menger MD, Giovanoli P, Vollmar B. Revascularization of skin grafts in a new in vivo model – HIF1alpha-mediated angiogenesis within the wound bed results in reperfusion of the graft capillaries. European Congress of Scientists and Plastic Surgeons (ECSAPS). Bern, 13.09.2008.
- Lindenblatt N, Calcagni M, Contaldo C, Menger MD, Giovanoli P, Vollmar B. Revascularisation of skin grafts in a new in vivo model-hypoxiamediated angiogenesis within the wound bed leads to reperfusion of the graft vasculature. 44th Annual Meeting of the Swiss Society of Plastic, Reconstructive and Esthetic Surgery. (SGPRAEC), Lausanne 04.10.08
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Collaborations:

- Prof. Dr. med. Michael D. Menger, Institut für Experimentelle Chirurgie, Universitätsklinikum, Homburg/ Saar, Deutschland
- Prof. Dr. med. Brigitte Vollmar, Institut für Experimentelle Chirurgie, Universität Rostock
- PD Dr. Guido A. Wanner, Klinik für Unfallchirurgie, Universitätsspital Zürich
- Dr. med. Jan Plock, Klinik für Plastische, Rekonstruktive und Aesthetische Chirurgie, Inselspital Bern
- Dr. med. Karl Lang, Institute of Experimental Immunology, Department of Pathology, University Hospital Zurich
- Prof. Dr. med. Simon P. Hoerstrup, Departement Surgical Research and Clinic for Cardiovascular Surgery, University Hospital Zürich
- Dr. med. Christian A. Schmidt, Clinic for Cardiovascular Surgery, University Hospital Zürich
- Dr. Eric P. Meyer, Institute of Zoology, University of Zürich
- Klaus Marquardt, Center for Microscopy and Image Analysis, University of Zürich

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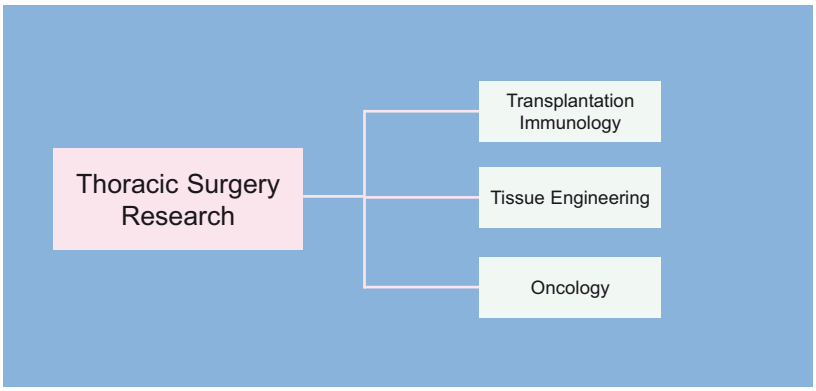
2.5 Thoracic Surgery Research


PD Dr. med.
Sven Hillinger


Prof. Dr. med.
Walter Weder



2.5.1 Transplantation Immunology


PD Dr. med.
Sven Hillinger


PD Dr. med.
Ilhan Inci


Dr. med.
Wolfgang
Jungraithmayr


Dr. rer. nat.
Stephan Arni


Dr. med.
Barbara Erne


Prof. Dr. med.
Stephan Korom

A mouse model of orthotopic, single-lung transplantation
W. Jungraithmayr, S. Korom, S. Hillinger and W. Weder

Given the unique interaction of the lung with the environment, experimental transplantation models that reflect neither the anatomic nor the physiologic peculiarities of this organ are inherently limited in their significance. Since the first orthotopic rat lung transplantation in 1971, much effort has been undertaken to modify, facilitate, and optimize this approach to develop a reproducible model. Currently, this method is well accepted and standardized for the investigation of immune-mediated and non–immune-mediated events that lead to rejection after lung transplantation. Although in recent years the application of the orthotopic rat and large-animal transplantation models contributed significantly to the elucidation of these mechanisms the scope of those investigations is limited by the scarcity of knockout and transgenic models. Ideally, mice offer a wide range of studying genetic modifications, and this has been utilized in employing models of subcutaneous/intra-abdominal tracheal implantation. However, ignoring target organ specificity (tracheal vs bronchioles), perfusion requirements (diffusion vs vascular circulation), and physiology (no ventilation vs regular ventilation) impairs the exploratory power of this approach. Orthotopic pulmonary transplantation in mice has been made difficult by the microsurgical demands, facing a diminutive situs with extremely fragile tissue. Although applying the basic principles of the rat transplantation technique, the procedure of lung transplantation in mice differs substantially in regard to particular technical aspects. Recently reported by Okazaki and colleagues for the first time, we describe our independently developed technique of orthotopic single-lung transplantation in the mouse. This publication focuses on the microsurgical characteristics of the procedure, with detailed discussion of key operative steps and their pitfalls. Extending the survival for up to 90 days, we report on the feasibility of a novel animal transplantation model, which may serve as a future pivotal tool for utilizing transgenic/knockout techniques in pulmonary engraftment.

C57BL/6 mice served as recipient, with Balb/c as donor. At time of harvest, explanted lungs were perfused with Perfadex, and the heart–lung block excised. Under 30 to 40× magnification, vessels and bronchus were cuffed. Following left thoracotomy in the recipient, hilar structures were incised and cuff-anastomosed with the corresponding donor parts. Allogeneic and syngeneic transplantations (n = 12/group) were performed with a follow-up period of 5 days and up to 90 days, respectively.

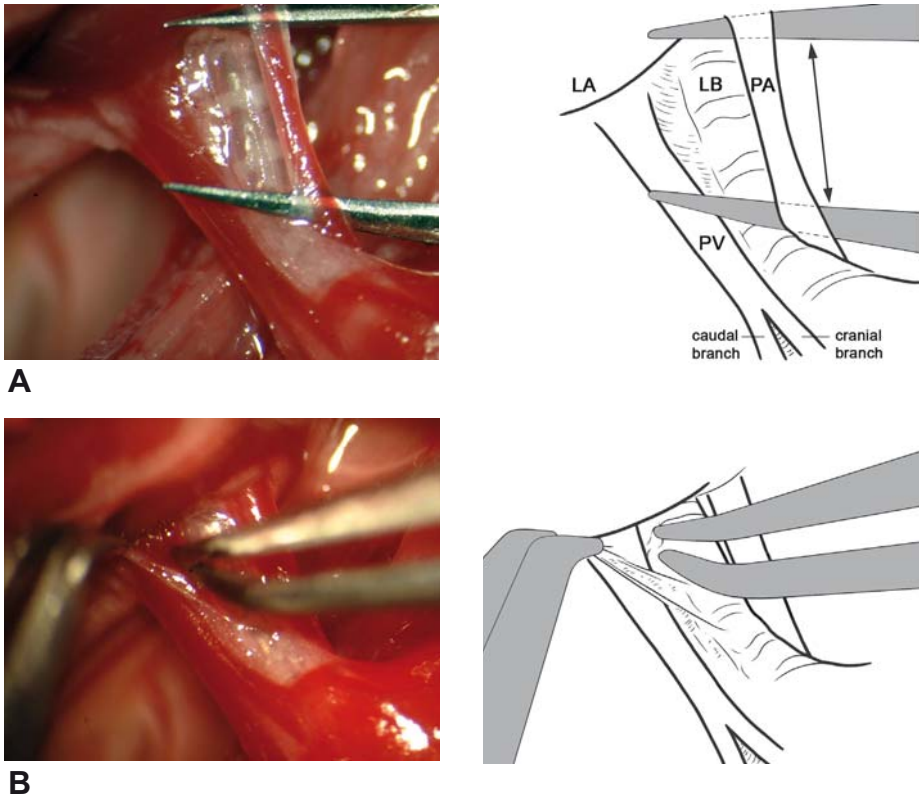


Figure 1 A, Isolation of the pulmonary artery is achieved by gentle spreading motions using the tips of the forceps. The pulmonary artery is relatively robust and resistant to shearing forces.
B, Separation between left bronchus and pulmonary vein is performed as close to the left atrium as possible. Preparation elsewhere invariably leads to injury and rupture of either left bronchus or pulmonary vein.

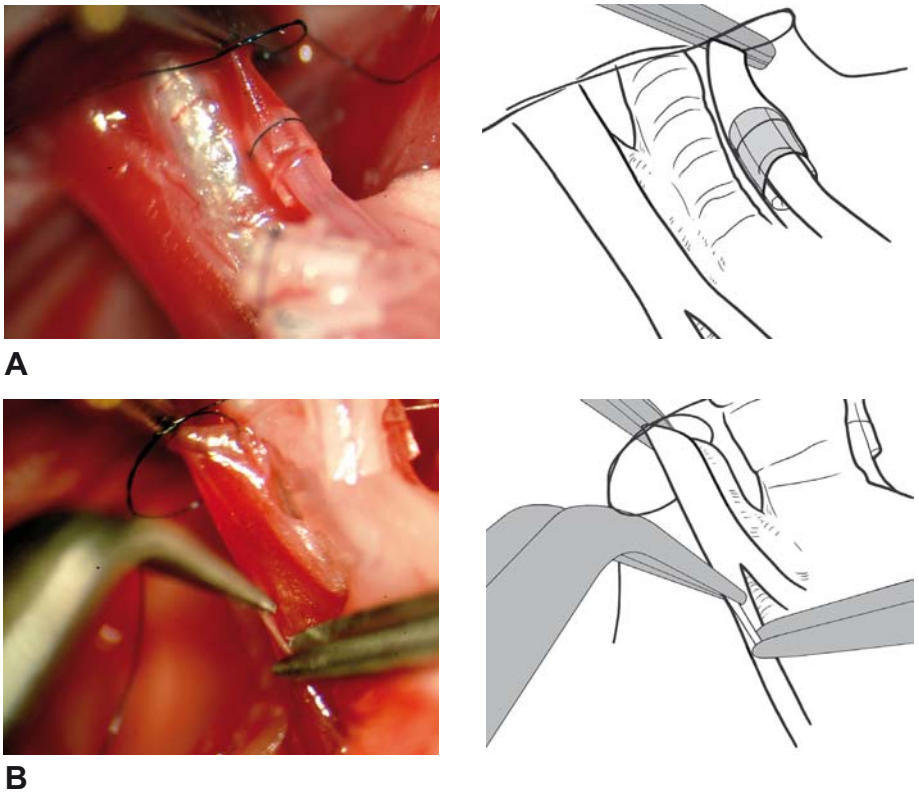


Figure 2 A, Once the cuffed donor artery is introduced into the recipient artery, it adheres inside the vessel without the aid of an additional clip.
B, The lower branch of the pulmonary vein is transversely incised; the upper branch is kept for a potential salvage procedure (in case of failure of the primary introduction).

The success rate of lung transplantation in mice was 87.5% (21/24). Mean cold ischemia time was 32.3 ± 3.7 minutes, and warm ischemia time was 30.8 ± 9.5 minutes. Deaths were due to bleeding during dissection of the hilus and/or caused by thrombosis postoperatively. Allogeneic grafts were rejected by day 5; syngeneic grafts were slightly congested but mainly unchanged up to day 90 posttransplantation.

Unilateral lung transplantation in mice can be performed in a standardized and controlled fashion with low mortality, comparable to the rat. Employing transgenic and knockout mice strains, this procedure holds great promise to advance the understanding of immunologic pathways in acute and chronic rejection in a physiologic model of pulmonary transplantation.

Experimental approach to chronic rejection of lung allograft in sensitized recipients

W. Jungraithmayr, S. Arni, S. Hillinger, S. Korom, W. Weder

Drawing from our previously reported pilot experiments (see annual report 2007), we successfully established an immunosuppressive regimen, based on RPM and CsA therapy, that induced pre-fibrotic lesions within small airways aequivalent to the initial phase of obliterative bronchiolitis. This regimen consisted of:

- RPM (0.5 mg/kg/d) from d -7 to d +21, followed by RPM (3x 0.5 mg/kg/wk) from d +21 to d +84
- CsA (2.5 mg/kg/d) from d 0 to d +7
- harvest at d +84

This treatment pattern successfully abrogated the leading immunological phenomenon of acute vascular rejection and allowed for a shift toward bronchial immune interaction. Under this regimen we observed a step-wise deterioration of respiratory bronchial epithelium (Fig. 1, A-D) with severe lymphocyte peribronchial infiltration and developing luminal fibroproliferative changes, replacing partially the respiratory epithelium leading to a narrowing of the bronchial lumen (Fig. 2, A-L). In these sections, positivity for α-SMA was shown, whereas collagen expression (sirius-red staining) was weak, indicating a developing fibrotic lesion.

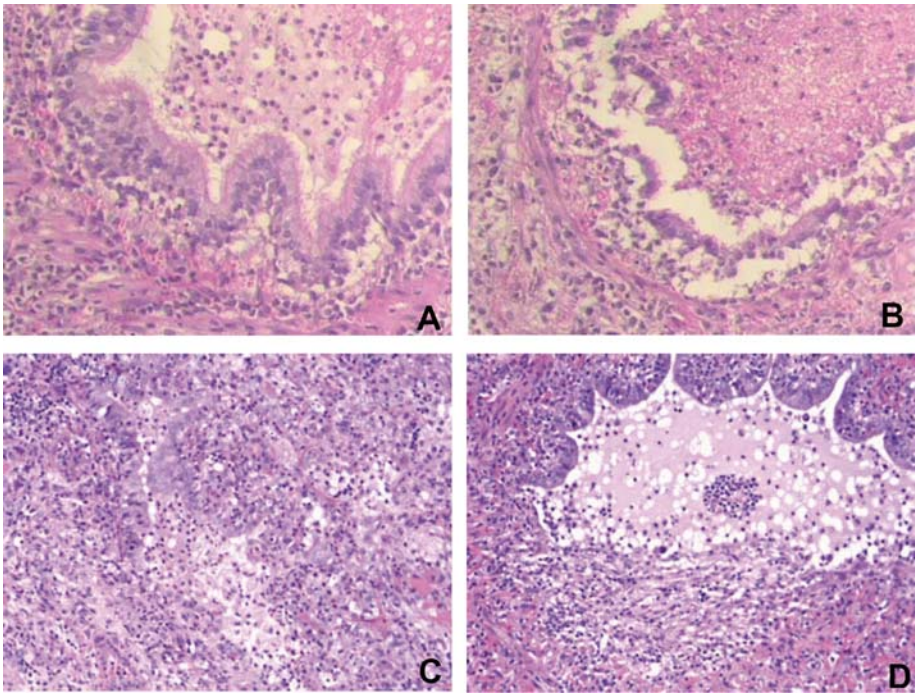


Figure 1, A-D: Histology (H&E) on paraffin sections in grafts on day 21 post-Tx (A), day 42 (B), day 70 (C) and day 84 (D) after allogeneic Tx from LBNF1 → sensitized LEW. Fibroproliferative changes displayed in Fig. D occurred in 3 animals (group: n=5). Within the section D, fibroproliferative changes were found in 80% of all bronchioles in whole lung specimens in this group. (Original magnification A, B: ×200; C, D: ×100).

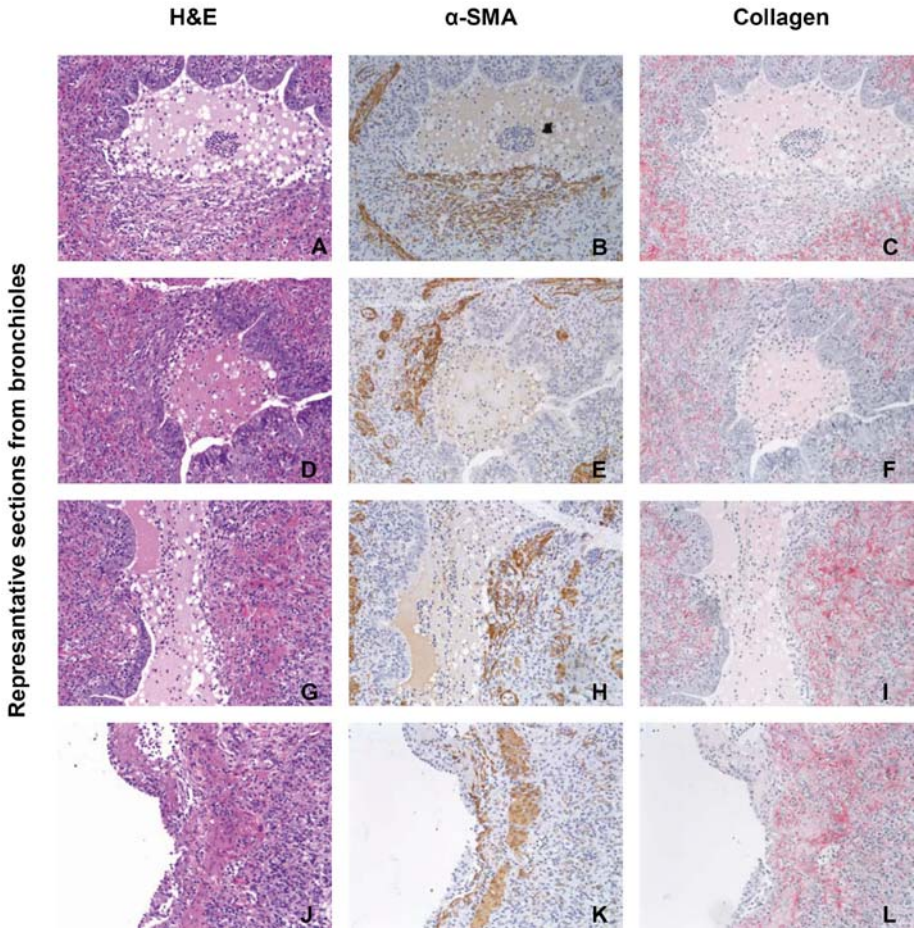


Figure 2, A-L: H&E-staining (A, D, G, J) and immunohistochemistry for α-SMA (B, E, H, K) and collagen (sirius red, C, F, I, L) in LBNF1 lung allografts, transplanted into LEW recipients, 84 days post-Tx. Images are randomly chosen representative sections from the same location. The primary antibody of α-SMA is detected using a horse-redish-peroxidase coupled antibody and DAB, the staining is identified as brown staining of myofibroblasts in bronchioles (B, E, H, K). Corresponding sections were stained for collagen by sirius red (C, F, I, L), (Original magnification A-L: ×100). Arrows indicate the site of loss of respiratory epithelium and the development of fibroproliferative changes.

Intragraft mRNA levels of IL-2, IL-10 and TBF-ß-1

In a first approach analyzing supportive data on the cytokines IL-2 and IL 10 and in order to correlate our findings, we were using RT-PCR in allografts and naïves on day 84 post-Tx. IL-2 was upregulated moderately whereas IL-10 was increased significantly over baseline on day 84 post-Tx. Also, we could detect TGF-ß-1 and found a relevant increase over baseline on day 84 post-Tx, indicating a certain degree of the presence of developing fibrosis (Fig. 1). Since regulatory T cells are a major source of IL-10 and TGF-ß-1, we suppose that a certain role is ascribed to regulatory T cells during the development of chronic rejection in orthotopic lung transplantation.

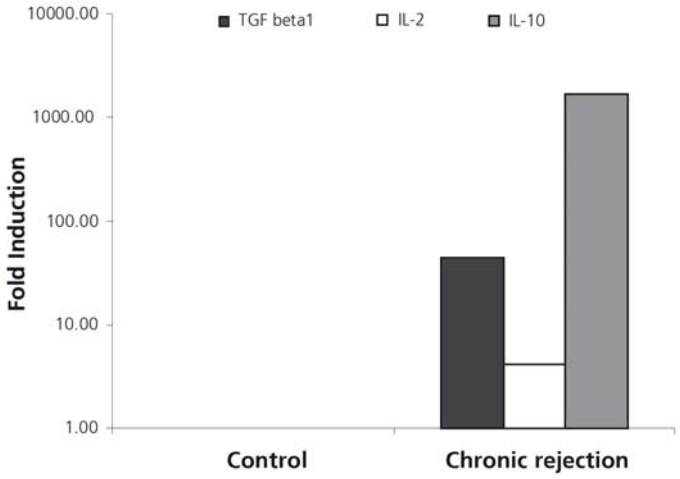


Figure 1: TGF-ß 1, IL-2 and IL-10 is upregulated on day 84 post-Tx in allografts from sensitized recipients undergoing established immunosuppression. Values are expressed as fold increased over baseline in controls. Values are representative for 2 experiments of one animal.

The transcription factors T-bet and GATA-3 in lung allograft rejection
W. Jungraithmayr, S. Korom, S. Hillinger, W. Weder, M. Hersberger

Recent research demonstrated that the differentiation of native Th-cells towards Th1 or Th2 is regulated by the upstream transcription factors T-box (T-bet) and GATA-binding protein 3 (GATA-3). T-bet promotes the synthesis of Th1-cytokines, while inhibiting lymphocyte differentiation into Th2-secreting cells. In contrast, GATA-3, a member of the GATA family of zinc finger proteins plays a pivotal role in the development of the Th2 phenotype while inhibiting Th1. These key transcription factors are crucial in determining Th-polarization. GATA-3 was found to increase IL-4 expression after G-CSF stimulation and suppressed AR in stem cell transplantation. T-bet expression was reduced in umbilical cord blood (UCB) compared to adult peripheral blood in decreased graft-versus-host disease (GVHD) after UCB transplantation. To date, research on the involvement of these two transcription factors in pathways of acute and chronic rejection are sparse. To correlate the impact of increased intragraft m-RNA expression of T-bet and/or GATA-3 with the expression of key cytokines in pulmonary rat transplants, we developed, in collaboration with the laboratory of PD Dr. Hersberger, Division of Clinical Chemistry and Biochemistry, University Children's Hospital Zurich, a real-time PCR assay. In a first proof-of-concept project during acute allograft rejection, we showed that the ratio of T-bet/GATA-3 increased, signifying a shift toward a Th1 environment during acute pulmonary rejection (LBNF1 to LEW) Accordingly, in syngeneic control animals and in native rats, the ratio was not shifted toward Th1 (Figure 1).

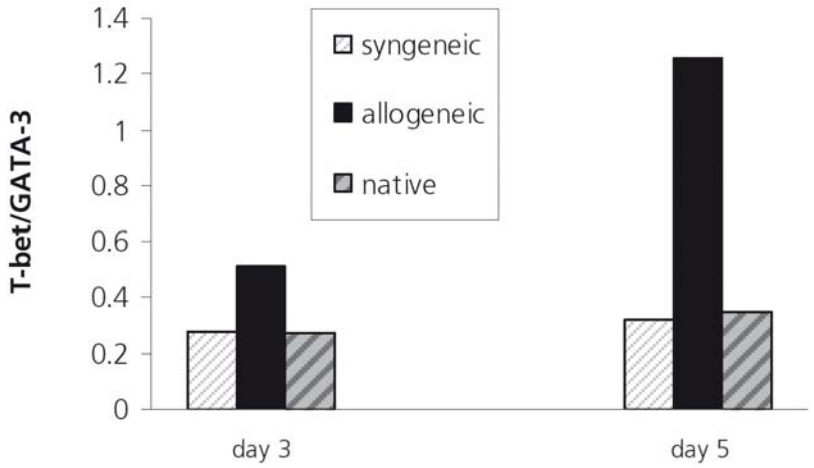


Figure 1: Allogeneic (LBNF1 → LEW), syngeneic (LEW → LEW) and naive (LBNF1) lung specimen at day 3 and 5 post-Tx in an acute rejection model; the increase in T-bet/GATA-3 ratio indicates a shift towards Th1 response; panel shows one out of four representative experiments (n=1/group).

Advancing our studies, we plan to assess the dynamic balance of Th1/Th2 in the development of pulmonary CR in analyzing intragraft T-bet and GATA-3 mRNA, and correlating these values to expression of pivotal intragraft cytokines.

Effect of N-Acetylcysteine on ischemia-reperfusion injury following lung transplantation

I.Inci, B.Erne S. Arni, S. Hillinger, B. Leskosek, W. Weder

Lung transplantation has become an effective therapeutic option in the treatment of patients with end-stage pulmonary diseases. However, early acute graft dysfunction continues to be a serious obstacle to successful lung transplantation, accounting for significant postoperative morbidity and mortality.

N-Acetylcysteine (NAC) is a precursor of the most important physiological antioxidant glutathione. Sulphydril-containing compounds, especially reduced glutathione (GSH), 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. GSH can also scavenge free radicals produced by oxidative challenges. There have, therefore, been many suggestions that reduced GSH may be useful therapeutically as an antioxidant and cytoprotective agent .

In this experimental study we wanted to investigate whether donor and recipient treatment with NAC would reduce ischemia-reperfusion injury following lung transplantation after 24 hours of cold ischemic storage in a pig left lung transplant model.

Left lung transplantation was performed in 12-paired pigs (25-35 kg). Group I: Lung transplantation without any treatment (Control group). Group II: Donor and recipient treatment with 150 mg/kg intravenous N-Acetyl-Cysteine. Donors were treated 1 hour before harvest. Recipients received the drug 1 hour before reperfusion as an intravenous bolus injection (150 mg/kg) followed by 12.5 mg/kg/hour continuous perfusion during the 8 hour observation period. Graft parameters: During the 8 hours of observation period hemodynamic measurements (heart rate, mean arterial pressure, mean pulmonary artery pressure, central venous pressure, cardiac output and pulmonary artery occlusion pressure), blood gas analysis of arterial and mixed venous blood, FiO2, tidal volume, minute volume, PEEP and peak inspiratory pressure were recorded every hour. Oxygenation was measured by means of PaO2/FiO2. Extravascular lung water (EVLW) level, a direct assessment of reperfusion edema, was measured. Serum was collected every 2 hours during the observation period for cytokine assessments. Left lower lobe was used for histologic examination.

We obtained a good oxygenation, less extravascular lung water index, low airway pressure in NAC treated group compared to control animals. The analysis of variance for repeated measures using all measurements at 10 time points made during 8 hours of observation (Baseline, 10 min. befor contralateral side occlusion, 10 min. after occlusion, 1,2,3,4,5,6, and 7 hour of reperfusion with transplanted lung) period differed significantly between the groups for mean pulmonary artery pressure, airway pressure, oxygenation (PaO2/FiO2) and Extravascular Lung Water Index (EVLWI) ($p<0.05$) (Figure 1,2,3, and 4). Assays for metabolic parameters and histologic assassment are pending.

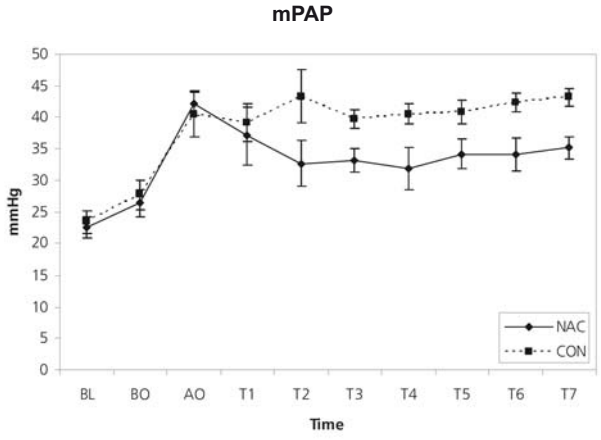


Figure 1. Mean Pulmonary artery pressure (mPAP). Time: BL: Baseline, BO: 10 before occlusion of the right lung, AO: 10 min. after occlusion of the right side, T1: 1 h after occlusion of the right side, T2: 2 h after occlusion of the right side, T3: 3 h after occlusion of the right side, T4: 4 h after occlusion of the right side, T5: 5 h after occlusion of the right side, T6: 6 h after occlusion of the right side, T7: 7 h after occlusion of the right side.

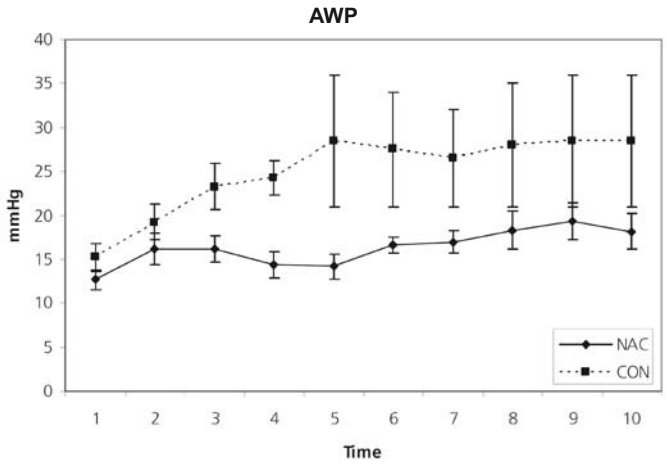


Figure 2. Airway pressure. Time: BL: Baseline, BO: 10 before occlusion of the right lung, AO: 10 min. after occlusion of the right side, T1: 1 h after occlusion of the right side, T2: 2 h after occlusion of the right side, T3: 3 h after occlusion of the right side, T4: 4 h after occlusion of the right side, T5: 5 h after occlusion of the right side, T6: 6 h after occlusion of the right side, T7: 7 h after occlusion of the right side.

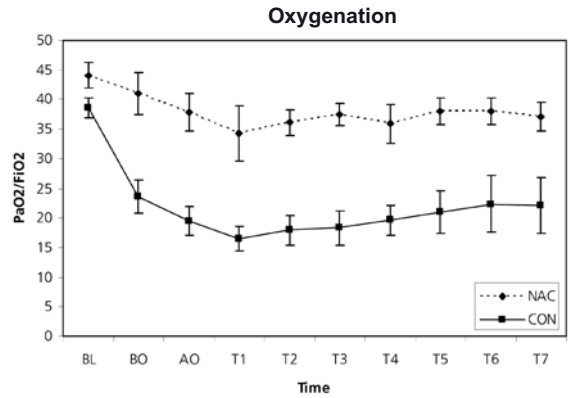


Figure 3. Oxygenation (PaO2/FiO2). Time: BL: Baseline, BO: 10 before occlusion of the right lung, AO: 10 min. after occlusion of the right side, T1: 1 h after occlusion of the right side, T2: 2 h after occlusion of the right side, T3: 3 h after occlusion of the right side, T4: 4 h after occlusion of the right side, T5: 5 h after occlusion of the right side, T6: 6 h after occlusion of the right side, T7: 7 h after occlusion of the right side.

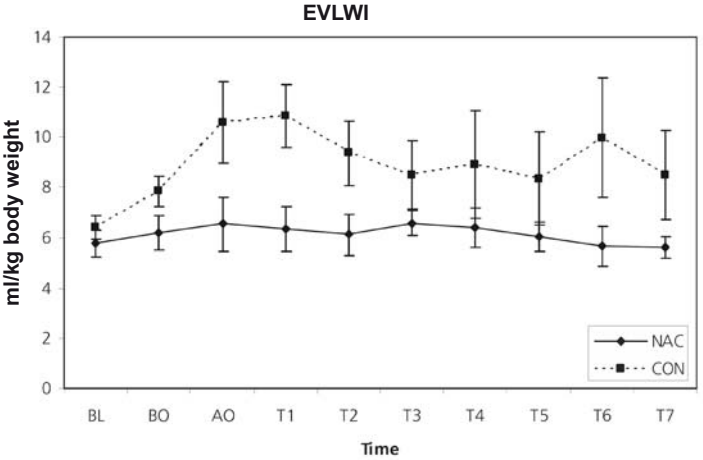


Figure 4. Extravascular lung water index (EVLWI). Time: BL: Baseline, BO: 10 before occlusion of the right lung, AO: 10 min. after occlusion of the right side, T1: 1 h after occlusion of the right side, T2: 2 h after occlusion of the right side, T3: 3 h after occlusion of the right side, T4: 4 h after occlusion of the right side, T5: 5 h after occlusion of the right side, T6: 6 h after occlusion of the right side, T7: 7 h after occlusion of the right side.

In this pig left lung transplant model we showed the superiority of NAC treatment attenuated ischemia reperfusion injury after 24 hours of cold ischemia following lung transplantation.

Achievements 2008

- Establishment of orthotopic mouse lung transplant model

Collaborations:

- Dr. I. De Meester, Prof. Dr. S. Scharpé, Department of Pharmaceutical Sciences, University of Antwerp, Antwerp, Belgium
- Dr. P. Vogt, Department of Clinical Pathology, University Hospital Zurich, Zurich, Switzerland
- Prof. Gesine Hansen, Christa Acevedo, Dr. Rau Gunnar, University Hospital, Hannover, Germany

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2.5.2 Tissue Engineering

Dr. med.
Qiang Tan

Dr.med.
Barbara Erne

Manfred Welti

PD Dr. med.
Ilhan Inci

In the year 2008, we translated our previous in-vitro studies on the in-vivo bioreactor design for the first time to sheep pilot experiments of tissue engineered trachea.
The sheep was laid on her belly right first after the anaesthesia and airway incubation connected to a ventilator. A slip-thickness skin graft, 10x10 cm in size, was harvested from the right foreleg of the sheep. Two reservoirs of an intravenous Port-system were then implanted subcutaneously on the neck with the catheters pulled forward to the front neck through an artificial channel made with a plastic probe (Fig.1). The sheep was than switched to the supine position where a cervical incision was made to expose 6cm long trachea. A 2 cm long, 1 cm wide window was made at the anterior cartilage part of the trachea. The defect was first covered with the split-thickness skin graft by interrupted sutures. Two arch shaped PEGT/PGT scaffold, pre-seeded with chondrocytes were then fixed to the remaining lateral cartilage tissue and the skin graft on the tracheal defect with interrupted sutures (Fig. 2). The two Port-catheters were inserted into the two PEGT/PGT scaffold respectively before an acellular porcine dermis matrix (Permacol) were used to close the whole tissue engineered trachea. The incision was closed layer by layer. Right after the operation two portable peristaltic pumps were connected to the implanted Ports respectively. One continuously delivered the medium into the implanted tissue engineered trachea with the other sucked the waste out. After sheep resumed from anesthesia, the pumps were set on the sheep back in a special jacket pocket to maintain the continuous medium perfusion. This in-vivo bioreactor design preserved for one week with the medium changed everyday and daily chondrocytes injection into the scaffold. The sheep survive for one and half month without dyspnea, stridor or any other inspiratory complications. The biopsy showed cartilage tissue formed inside the PEGT/PGT scaffold and an intact epithelial layer cover the whole inner lumen surface. The skin graft keratinocytes were replaced by tracheal eptielia (Fig. 3).

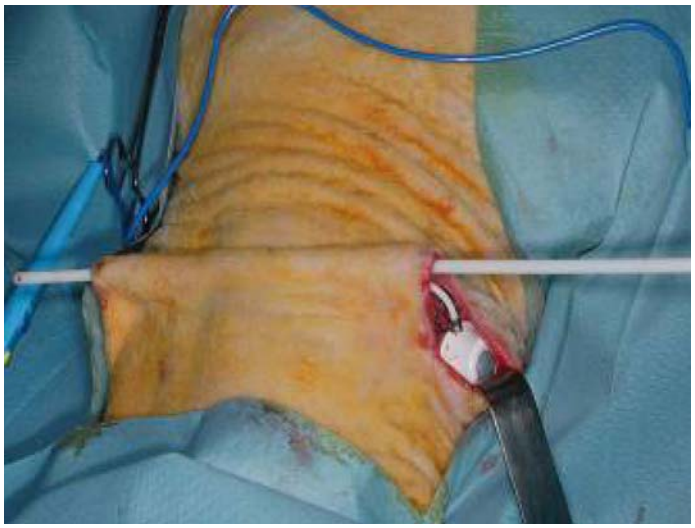

Fig.1 Port implantation subcutaneously on the back of the neck with a channel to the front.

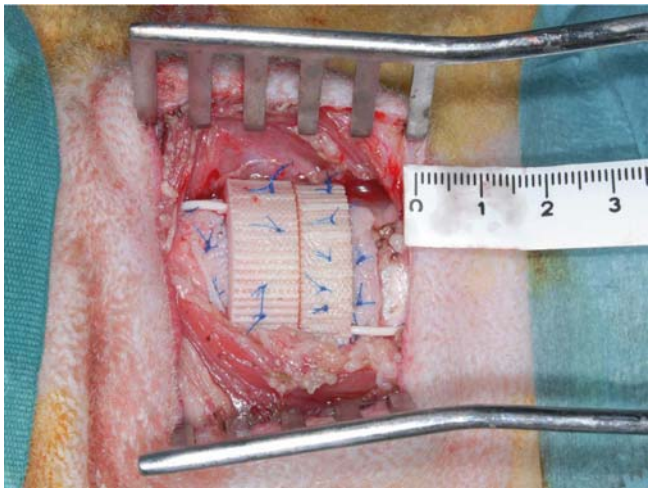

Fig. 2 The tracheal defect was closed by tissue engineered trachea with two Port-catheters inserted.

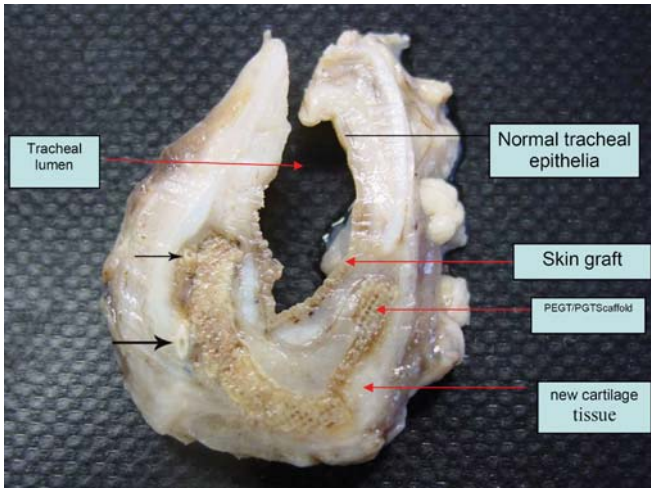

Fig 3. Autopsy showed cartilage tissue formed in the PEGT/PGT scaffold and normal tracheal epithelia cover the skin graft.

Achievements 2008

- First large animal experiments

Collaborations:

- Dr. L. Moroni, Twente Univeristy, IsoTis S.A., Bilthoven, Netherlands
- Prof. Clemens A. van Blitterswijk, Departement of Tissue Regeneration University of Twente, Enschede/Netherlands.
- Prof. Donat Spahn, Department of Anaesthesia, University Hospital Zurich, Zurich/ Switzerland
- Dr. Claudio Contaldo, Department of Plastic, Reconstructive and Hand Surgery, University Hospital Zurich, Zurich/ Switzerland.
- Dr. Rudolf Steiner, Department of Oncology, University Hospital Zurich, Zurich/ Switzerland
- Dr. Ashraf Mohammad El-Badry, Department of Visceral and Transplant Surgery, University Hospital Zurich, Zurich/ Switzerland

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2.5.3 Oncology


PD Dr. med.
Sven Hillinger


PD Dr. med.
Isabelle Opitz


Dr. phil.
Stephan Arni


Dr. med.
Barbara Erne


PhD student
Thomas Wiedl


Dr. med.
Stephane Collaud


Manfred Welti


Monica Desantis

Activity based protein profiling (ABPP) of human lung adenocarcinoma biopsies

T. Wiedl, S. Arni, S. Hillinger, W. Weder

Lung cancer is the most diagnosed form of cancer and the major cause of cancer-related deaths with a worldwide mortality of 1.3 million in 2007. Patients suffering from lung cancer have poor prognosis and face a 5-year survival rate of 10% to 15%. Non-small cell lung carcinoma (NSCLC) accounts for 80% of all lung malignancies, 40% of which are adenocarcinomas representing the most common lung cancer subtype. The “TNM Classification of Malignant Tumors” staging system remains the method of choice for determining lung cancer prognosis. Thereby it has become evident that predicted and real outcomes can vary significantly, also for patients with same stage of disease. Thus establishing predictive molecular markers is of utmost importance. Although effort has been undertaken to link protein- or gene-expression profiles of lung adenocarcinoma biopsies with treatment response or survival, no marker with a significant predictive clinical utility is available so far.

One explanation for this unsatisfactory situation is that detected changes in protein- or gene-expression profiling experiments are based on measuring abundances instead of real enzymatic activities. This becomes important when considering that mRNA and corresponding protein levels do not necessarily correlate. The same holds true for mRNA transcripts and enzymatic activities. Therefore protein- and gene-expression profiling methods might fail to detect crucial changes in enzymatic activities caused by posttranslational events during tumor progression and treatment response (Sieber et al.). The serine hydrolase (SH) superfamily for example comprises a large and diverse repertoire of enzymes that make up 1% of the human proteome and have previously been linked to cancer and lung cancer. Distinct serine hydrolases temporarily exist in inactive enzyme precursor forms or are regulated by endogenous inhibitors, all of which are posttranslational events that directly influence enzymatic activity. In order to address this problem, Professor B. Cravatt and colleagues developed a new technique termed `activity based protein profiling` (ABPP) (Liu et al.) that allows determination of activity profiles of serine hydrolases and distinct other enzyme families in cell lines and biopsies. This is achieved by employing activity based probes (ABPs) that selectively bind to active sites of solely active enzymes, thereby making a direct readout of activity profiles in a given proteome possible. Quantitative data is then acquired by measuring emitted fluorescence during a gel-based phase by making use of an fluorophor tagged ABP. Qualitative information is obtained through mass spectrometry (MudPIT) experiments during a gel-free phase by analyzing representative proteomes that have been labeled and enriched using biotin tagged ABPs.

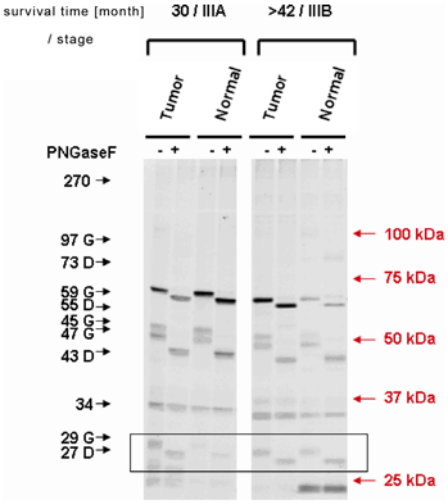


Figure 1: Serine hydrolase activity profiles of lung adenocarcinoma biopsies determined by one-dimensional sodium-dodecylsulfate polyacrylamide gel electrophoresis (1D-SDS-PAGE). Membrane fractions of normal and corresponding malignant biopsies of two patients at comparable stage (IIIA and IIIB), but with different overall survival times (30 months and >42 months) were screened for serine hydrolase activities. Bands within the box represent differences in Cathepsin G activities in biopsies derived from the two patients. PNGaseF was used for protein deglycosylation (glycosylated, “G” and deglycosylated, “D”).

			30 / IIIA		>42 / IIIB	
	M_w [kDa]		Tumor	Normal	Tumor	Normal
	glycosylated	deglycosylated	[spectral counts]	[spectral counts]	[spectral counts]	[spectral counts]
Fatty acid synthase	-	270	282	98	21	109
Seprase	97	73	60	-	43	5
Isoform 2 of liver carboxylesterase 1 precursor	59	55	1113	1137	141	1573
Arylacetamide deacetylase-like 1	47 / 45	43	163	159	68	77
α/β-Hydrolase domain containing protein 10	-	34	117	51	65	53
Cathepsin G precursor	29	27	166	10	-	9

Figure 2: Qualitative and semi-quantitative analysis of serine hydrolase activities in lung adenocarcinoma biopsies. Mass spectrometry was employed for qualitative and semi-quantitative analysis. Spectral counts indicate enzymatic activities. Molecular weights of targeted serine hydrolases were determined using fluorescently labeled protein standards (column “Mw [kDa]”).

The aim of this project is to determine activity signatures of serine hydrolases in approximately 80 pairs of fresh-frozen human lung adenocarcinoma biopsies and corresponding normal lung tissues. Combined with clinical follow-up data, we hypothesize that the found activity patterns bear predictive potential and will ideally allow the discrimination of low-/high-risk lung adenocarcinoma patients.

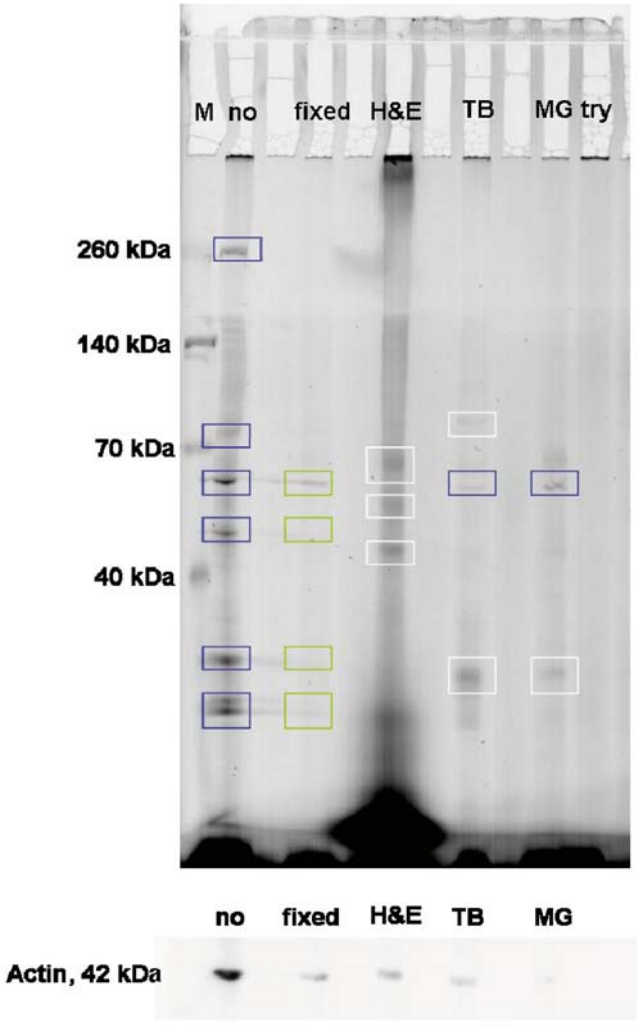
Laser-capture microdissection (LCM) as a further tool applied to activity-based protein profiling (ABPP) of lung adenocarcinoma

S. Collaud, T. Wiedl, S. Arni, S. Hillinger, W. Weder

Aim: LCM is a powerful tool to study inhomogeneous population of cells in –omics sciences. Its compatibility with ABPP proteomics has to be assessed.

Material and method: The compatibility of ABPP with different staining protocols for LCM used in conventional proteomics (H&E, Toluidin blue, Methyl green) were assessed on fresh frozen lung adenocarcinoma from the Zurich University Hospital tumor bank.

Results: Protein activity is impaired when conventional staining protocols were used.



Next steps: Once the staining protocols will be optimized for downstream activity-based proteomics, population of stroma and tumor cells of lung adenocarcinoma will be studied.

Immunotherapy for lung cancer

S. Hillinger, S. Arni

1) Establishment of methods to determine adenoviral titers

We started the production of our CCL19 and WT control viruses and of two more adenoviruses (mIL-7 and the control WT viruses) that we both received as a gift. All of our stocks were first amplified without knowing the exact title (for mIL7 and control WT) or from transfection of HEK-293 cells with the pAdHM15RGD-CCL19 and pAdHM15RGD wild type plasmid starting with low level title following the protocol of Luo et al [1]. For this reason we needed to establish routine methods to determine viral title (see below). To infect primary culture of dendritic cells we need to determine the optimal multiplicity of infection (MOI) to apply. Amount of viruses loaded per cells should be sufficient to cause a significant increase in the production of the cytokine/chemokine before injection of the infected DC cells in the tumor bed of the mouse lung cancer tumour. We need an assay to quantify the amount of viral particles present in our infectious media.

a) Plaque assay to measure the viral titer

We first started to establish this method. A monolayer of HEK 293 cells was seeded in each well of a 6 well plate. Then dilution of the viruses to be tested were prepared and incubated with the monolayer during 60 min at 37 C. After removal, the viral dilution were replaced with a mixture of nor mal media and warm agarose. Cells were then incubated for one week until plaque in the monolayer appeared. A formula based on the number of plaque formed is related to the dilution with the viral stock to be tested. In our hand this method did not work since an intact monolayer of cells after recovering with agarose was never achieved.

b) End point dilution to measure the viral titer

In this method was successful in our hand. We are now able to quantify each viral stock we produced.

2) Production of adenoviruses in HEK-293 cells

Finally amplification of the pAdHM15RGD-CCL19, pAdHM15RGD, mIL7 and control WT was successful since as of today we obtained:

40 ml of supernatant for mIL-7 at 4.1 x $10E^{+9}$ pfu/ml,

40 ml of supernatant for control for mIL-7 at 2.2 x $10E^{+8}$ pfu/ml,

20 ml of culture supernatant for AdHM15RGD-CCL19 at 3.5 $10E^{+7}$ pfu/ml,

20 ml of culture supernatant for AdHM15RGD at 2 $10E^{+7}$ pfu/ml.

Since the multiplicity of infection (MOI) needed to infect our dendritic cells are around MOI 0.1 to 10 we can theoretically start to infect dendritic cells with our preparation. We also observed some morphological changes in HEK293 cells infected with the AdHM15RGD-CCL19 and AdHM15RGD. Before lysis and detachment cells infected with those viruses were forming tumor like structures (see Figure 1)

HEK 293 infected with similar titer (i.e MOI=2) of different viruses do not seems to have the same kinetic of infection.

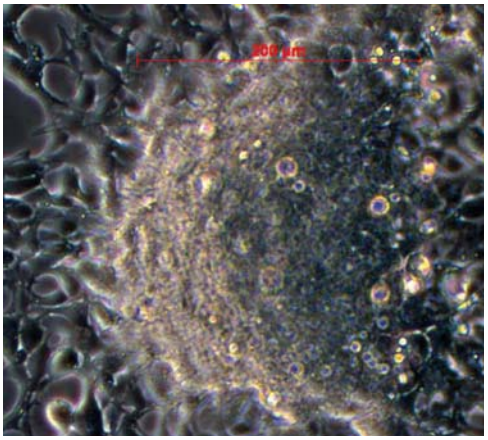


Figure 1: Tumour like structures formed in monolayers of HEK 293 cells infected with the RGD pseudotyped adenoviruses.

In Figure 2, the cells infected with mIL7 type viruses (a virus not pseudotyped with the RGD sequence) detach from the dishes faster as cells infected with AdHM15RGD-CCL19. Since the control viruses (WT for mIL7 and AdHM-15RGD) do behave similarly, our current hypothesis is that this may be due to the RGD sequence present in the surface of AdHM15RGD-CCL19 and AdHM-15RGD rather than the cytokine/chemokine they produce.
This is an unexpected but important point we will need to analyse further when we will infect primary culture of DC.

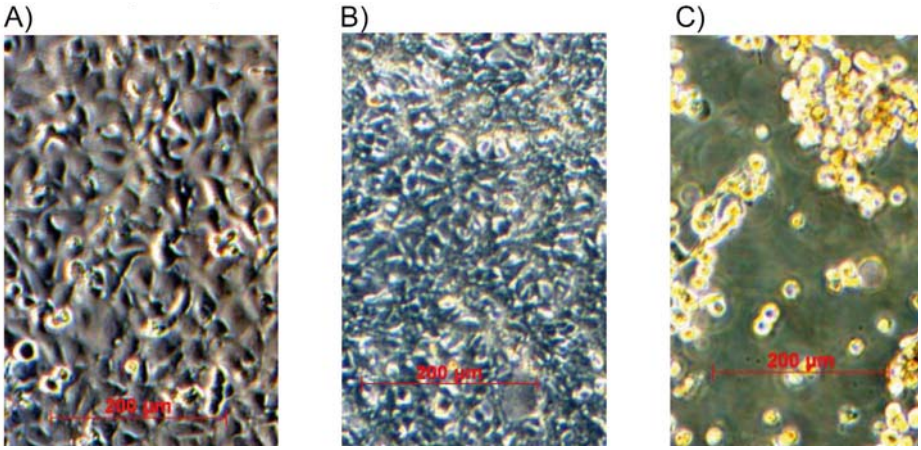


Figure 2: All are HEK-293 cells. A) Cells before adding adenoviruses. B) Are HEK293 cells at MOI 2 with AdHM15RGD-CCL19 at day 5. C) Are HEK293 cells at MOI 2 with mIL7 at day 5.

We hypothesize those difference in time of cell lysis may be due to the RGD sequence present in the surface of AdHM15RGD derived viruses

3) Establishment of a bioassay for mIL7
The murine cell line 2E8 (ATCC catalog No TIB-239) is a lymphoblastic B cell line. This cell line is dependant on mIL7 and can be used as a bioassay for the quantitation and detection of mIL-7. We expanded this cell line and freeze down stocks of cells. At the moment we tested the survival of this cell line with decreasing amount of IL7 in order to obtain a dose response curve.
The final goal is to plate 2E8 cells in 96 wells plate and expose them to increasing amount of the supernatant obtained after infection of the HEK AD293 cells with the mIL-7 adenoviruses.

We will also need to UV irradiate the culture supernatant in order to minimise the risks of cell killing by the viral infection. We plan to measure the survival of 2E8 cells in IL7 containing media with the MTT colorimetric assay.

4) Establishment of a bioassay for mCCL19
The chemokine CCL19 do have a chemotactic effect on activated T cells and allow the migration of those cells through a chemotactic gradien. We will use T and B cells collected from the spleen of mouse to measure the chemotactic power of UV irradiated culture supernatant of HEK293 infected cells.

Achievements 2008

- SNF-grant 'Immunotherapy for lung cancer'
- Grant: Award Sophien-Stiftung 2008
- Grant: Dr. U. Arnold and Susanne Huggenberger-Bischoff
- Stiftung zur Krebsforschung

Collaborations:

- Prof. S.M. Dubinett, Director of the UCLA Lung Cancer Program, Dr. S.Sharma, Associate Research Professor, University of California Los Angeles
- Prof. S.M. Dubinett, Director of the UCLA Lung Cancer Program, Dr. S.Sharma, Associate Research Professor, University of California Los Angeles
- Prof B.Cravatt, Scripps Institute, San Diego
- Prof R.Aebersold, Institute of Molecular Systems Biology, ETH Zürich
- Prof. H.Moch and Dr. A.Soltermann, Department of Pathology, USZ

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Prognostic Marker for Malignant Pleural Mesothelioma

I. Opitz, A. Schramm

Patients with a malignant pleural mesothelioma (MPM) have even with an aggressive therapy a poor survival. Prognostic marker would help to select patients for different treatment concepts. One important oncogenic pathway involves ß1-integrin and EGFR signalling to p27. ILK (integrin linked kinase), periostin and p21 are also parts of this pathway. We wanted to investigate the relevance of this axis in patients with a MPM.

Quadruple punches of 352 MPM were studied for the immunohistochemical expression of EGFR, p27, p21, ILK, periostin and ß1-integrin using respective antibodies. Staining intensity was semi-quantitatively scored (0-3) summed up and divided by 4 to give a global score. This global score was correlated to overall survival and histological subtype.

Clinical data from 206 patients was available. 31% of the mainly male patients got any therapy. The histological subtypes were 31% epitheloid, 17% sarcomatoid and 52% biphasic.

Increased EGFR (p=0.0002), ß1-integrin (in membrane and core) expression (p=0.03), p27 (p=0.02), periostin (p=0.0002) were significantly more frequent in the epitheloid subtype, whereas no preference for a particular subtype was found for ß1-integrin (in the stroma) (p=0.59), p21 (p=0.23) and ILK (p=0.40).

For the 126 patients with complete follow-up data survival time was correlated with protein expression. The median survival time was 11.7 months.

Histology (epitheloid versus sarcomatoid versus biphasic) (p=0.01), ther-apy (yes versus no therapy) (p=0.001), age (<62 years versus >62 years) (p=0.02), protein expression of p27 (low versus high) (p=0.02), p21 (no expression versus expression) (p=0.006) and ILK (no expression versus expression) (p=0.02) were significant prognostic factors for longer survival in the univariate analysis.

In this large TMA based tissue bank study EGFR, ß1-integrin, p27 and periostin seem to be diagnostic marker for epitheloid global histological type. Independent prognostic marker for better overall survival were histology, therapy, age, protein expression of p21, 27 and ILK.

PTEN expression is a strong predictor of survival in mesothelioma patients.

I. Opitz, A. Schramm

Malignant pleural mesothelioma (MPM) is a highly aggressive tumour with poor prognosis and limited response to therapy. MPM is characterized by complex chromosomal aberrations, including chromosome 10 losses. The tumour suppressor gene PTEN located on chromosome 10q23 plays an important role in different cancer, but it's relevance for MPM is unclear. All malignant mesotheliomas, diagnosed between 1975 and 2004, were retrieved from the archives of the Zurich Pneumoconiosis Research Group, Switzerland. The total of 341 cases comprised 112 epithelioid, 183 biphasic and 46 sarcomatoid types. The tissue specimens were mainly derived from postmortem examination (77% autopsy, 23% biopsy) and had uniformly been formalin-fixed and paraffin-embedded. They had all been originally examined and classified for the histological subtype by one experienced lung pathologist and were reviewed to identify suitable areas for tissue microarray construction. The construction of a set of three tissue microarrays (TMA) was accomplished with a custom-made, semiautomatic tissue arrayer (Beecher Instruments, Sun Prairie, WI, USA) as described. Clinical data from 206 patients were available. 105 patients were stage T4 and 92 patients presented with regional and mediastinal lymph node metastasis. Loss of PTEN expression was observed in 62% of the cases. The survival time was correlated to PTEN expression in 126 cases with complete follow-up data. Comparing any PTEN expression versus no expression, median survival time was significantly longer (p= 0.0001) in patients with PTEN expression (15.5 months; 95% CI: 3.8; 27.2 versus 9.7 months; 95% CI: 7.9; 11.7). Cox-regression analysis revealed an association between PTEN expression and survival (p=0.003) independently from the histological subtype (p=0.7). PTEN is an independent prognostic biomarker in mesothelioma patients. The frequent loss of expression of the tumour suppressor gene PTEN suggests involvement of the PI3K-AKT/protein kinase B (PKB) pathway in MPM, which may be relevant for future mesothelioma treatment.
PTEN and other marker will be assessed in our prospective database of patients that underwent induction chemotherapy followed by extrapleural pneumonectomy.

The value of ERCC1 as a prognostic marker for Malignant Pleural Mesothelioma

I. Opitz, A. Schramm

Expression of the excision repair cross-complementation group 1 (ERCC1) protein predicts response to platinol-based chemotherapy and survival in lung cancer patients. The relevance of ERCC1 expression in Malignant Pleural Mesohtelioma (MPM) has not yet been studied.

Three tissue microarrays (TMA) with biopsies of 356 MPM patients without standardized treatment were used as retrospective training set for immunhistochemistry of ERCC1. Staining intensity was semi-quantitatively scored (0-3) and percentage of ERCC1 positive cells (0-100%) was measured. A final H-score was calculated and correlated to overall survival of this retrospective data. From May 1999 to January 2009, 160 were intended to be treated with induction chemotherapy (42% cisplatin/gemcitabine; 58% cisplatin/pemetrexed) followed by extrapleural pneumonectomy (EPP). Response to chemotherapy according to modified RECIST criteria was available for 92 patients. One TMA with tumour of 107 MPM patients who underwent induction chemotherapy followed by EPP was constructed. Another TMA with 47 patients where pre-chemotherapy biopsies were available was constructed. ERCC1 expression was assessed and correlated to prospectively documented data. The influence on overall survival and response to chemotherapy were evaluated.

ERCC1 was expressed in 80% of the MPM in the retrospective series. Median survival of patients with ERCC1-H-score 260 was 8.8 (95% CI 7.1; 10.5) in comparison to patients with H-score³ 260 15.5 months (95% CI 8.0; 22.9). Cox-regression analysis revealed that ERCC1 H-score was the only independent marker for overall survival. Out of 92 treated patients with response assessment by modified >RECIST criteria, partial response was found in 30 patients, stable disease for 34 patients and progressive disease for 28 patients. Patients with an objective response to chemotherapy according to modified RECIST criteria was associated with a better survival (p=0.013). The median overall survival of all 160 patients was 19 months, of the 100 patients undergoing EPP 22 months. The patients undergoing EPP with low ERCC1 expression (dichotomized at the median value of 200) - assessed in the biopsy before chemotherapy - had a shorter survival with 13 months in comparison to 22 months survival of patients with a high expression of ERCC1 (p=0.057). A significant prognostic factor was the response to chemotherapy assessed by modified RECIST criteria: operated patients with progressive disease had a median survival of 15 months, in comparison to 23 months for stable disease as well as partial response (p=0.03).

Loss of ERCC1 expression was shown to be an independent prognostic marker for poor overall survival of mesothelioma patients without standardized treatment. In patients group undergoing multimodality treatment ERCC1 expression and response to platinol-based chemotherapy assessed by modified RECIST criteria seem to influence overall survival.

Achievements 2008

- Krebsliga grant

Collaborations:

- Department of Oncology (Emmanuela Felley-Bosco, Rolf Stahel)
- Department of Clinical Pathology (Alex Soltermann, Holger Moch, Peter Vogt)
- Institute for Biostatistics (Valentin Rousson, Burkart Seifert

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Malignant pleural mesothelioma –intrapleural therapy after surgery

Isabelle Opitz, Luca Ampollini, Stephan Arni

Malignant pleural mesothelioma is an aggressive tumour with increasing incidence that is expected to peak in the next two decades. The management of these patients is still controversial, with currently the best survival data after multi-modality treatment including induction chemotherapy with cisplatin and pemetrexed, surgery and radiotherapy. Nevertheless, local recurrence of the tumour remains a major problem. Intrapleural therapy is an attractive treatment option for local tumour control with promising results in early clinical and experimental studies but further refinement is still necessary. Beside chemotherapy, another approach for improved local tumour control is the intrapleural application of different immunomodulating substances. One particularly promising approach is to stimulate innate immunity. Toll-like receptor (TLR) belong to the family of pattern recognition receptors (PRR) and ligation of these receptors by conserved motifs of microorganisms (pathogen-associated molecules) results in activation of the innate immune response. TLR9 ligands bind unmethylated CpG clusters. Both bacterial DNA and synthetic unmethylated CpG oligonucleotides have been shown to enhance cellular and humoral immunity against cancers via TLR-9. Furthermore experimentally CpG-ODNs were found to be potent enhancer of chemotherapy and radiotherapy and there-fore might also qualify for multimodal treatment in mesothelioma. In the underlying study we wanted to assess the effect of intratumoral injection of immunomodulatory agents + plus intrapleural chemotherapy loaded to a fibrin sealant (Vivostat®) on the volume and the incidence of tumour recurrence.

Recurrence model: A tumour cell suspension of 50 µl $1x10^6$ rat malignant mesothelioma cells was inoculated subpleurally. Six days after inoculation, a tumour nodule of about 5mm in diameter was resected and animals were treated according to randomization after left-sided pneumonectomy and pleural abrasion: control (n=6), 500g CpG-ODN (Cytosine-phosphate-guanosine-oligodeoxynucleotide) (n=6), Cisplatin-Vivostat® (n=6), Cisplatin-Vivostat®+500g CpG-ODN (n=6).

Primary endpoint was the volume of tumour recurrence 6 days after treatment.

Secondary endpoints were the SRY-gene (sex-determining-region Y) expression for quantification of the ratio host/tumour cells into the local recurrence and cytokines expression profile in the tumour tissue by qPCR. Treatment-related toxicity was assessed by repeated blood samples. The volume of tumour recurrence was significantly reduced from $610mm^3$ in the control group to 11.7mm3 in the Cisplatin-Vivostat® group (p=0.005) and to 21.8mm3 in the Cisplatin-Vivostat®+CpG group (p=0.003). The determination of SRY gene by qPCR-technique showed a higher ratio host/tumour cells in the Cisplatin-Vivostat®+CpG group (45/55%) compared to the Cisplatin-Vivostat® group (27/73%). Pro-inflammatory cytokines (IFN-gamma, IL-6, IL-12) were increased after treatment with Cisplatin-Vivostat®+CpG group. No significant treatment-related toxicity was observed.

Adjuvant treatment with chemo- and immunotherapy lead to significant reduction of mesothelioma recurrence after surgery in this aggressive tumour rat model. An additional effect of immunotherapy might be the recruitment of inflammatory cells at the site of tumour growth and concomitant cytokines secretion.

Achievements 2008

- Krebsliga grant, ESMO Award

Collaborations:

- Department of Oncology (Emanuela Felley-Bosco, Rolf Stahel)
  Department of Clinical Pathology (Alex Soltermann, Holger Moch, Peter Vogt)
- Institute for Biostatistics (Valentin Rousson, Burkart Seifert)
- Department of Radiooncology (Andreas Hollenstein, Martin Pruschy)

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Identification of cells with stem cell/self renewal properties in malignant pleural mesothelioma

Claudia Frei, Isabelle Opitz, Emanuela Felley-Bosco

Mesothelioma (MPM) tumorigenesis is associated with asbestos fibres in the pleural space causing a chronic tissue repair. It is a devastating disease with a rapidly fatal outcome. The aim of our study is to identify cancer stem cells which could specifically be targeted for treatment.

By investigating the Sonic Hedgehog pathway we found a significantly increased Gli-1 expression in MPM tumors compared to normal pleura, indicating that stem cell signaling is active in MPM tumors. The stem cell signaling was maintained in primary MPM cell cultures since cyclopamine but not tomatidine could inhibit cell growth and Gli-1 expression.

To identify the stem cell component of tumors we used a functional approach based on the ability of cancer stem cells to efflux Hoechst33342 ("side population" (SP)). Using this functional approach we were able to isolate a SP from ZL55 mesothelioma cells. Sorted ZL55 SP gave rise to a SP and a non-side population (NSP), suggesting that the SP includes cells with self-renewal properties, whereas the ZL55 NSP gave rise only to a NSP. Similar results were obtained for two primary mesothelioma cultures. By characterizing the ZL55 SP and NSP we found an increased expression of ABCG2, a drug transporter responsible for the SP phenotype, and the stem cell maintenance gene Sox2 in the SP compared to NSP. This phenomenon was accompanied by a decreased expression in SP of differentiation markers mesothelin and N-cadherin.

Taken together these results indicate that cells with stem cell renewal properties are present in mesothelioma.

Outlook: Currently a sensitive tumorigenic xenograft in NOD/SCID mice is set up.

Functional inactivation of NF2/merlin in human mesothelioma

Claudio Thurneysen, Isabelle Opitz, Emanuela Felley-Bosco

The tumor suppressor merlin is encoded by the neurofibromatosis type 2 gene (NF2) which is located on chromosome 22q12 and mutations in this gene have been found in 40% of mesothelioma. Mutations including deletions and insertions lead to truncated and inactivated merlin. Experimental animal models indicate that disruption of the NF2 signalling pathway, together with a deficiency in ink4a, is essential for mesothelioma development.

Our hypothesis was that in human mesothelioma without detectable NF2 mutations, regulators of NF2/merlin activity such as CPI-17 would be altered. CPI-17 is an oncogene inhibiting the NF2/merlin phosphatase which is necessary to maintain NF2/merlin activity. Samples obtained from 44 mesothelioma, 3 asbestosis patients and 6 normal pleura from non-asbestos related disease patients were analyzed. Truncated NF2 transcripts or presence of isoform II only were observed in 11 mesothelioma samples. In all other mesothelioma samples only NF2 isoform I or isoforms I and II were detected. 18 mesothelioma and 1 normal pleura samples also expressed splicing variant delE2/3.

Unexpected variants in addition to wild-type were identified in 24 mesothelioma samples. NF2 protein was either truncated or phosphorylated on Ser 518 in primary cultures derived from 25 tumors. CPI-17 expression was significantly increased in tumor samples without deleted NF2 compared to normal pleura and tumor expressing truncated NF2. Our results support the hypothesis that the disruption of NF2 signalling is essential for the development of human mesothelioma. In tumors where no NF2 truncation can be detected, NF2 is rendered inactive by phosphorylation of Ser 518 and this can be explained at least in part by an increased expression of CPI-17.

Treatment with Cisplatin-Pemetrexed induces senescence pathways in malignant pleural mesothelioma tumor samples.
Roy Sidi, Isabelle Opitz, Emanuela Felley-Bosco

Malignant pleural mesothelioma (MPM) is an aggressive tumor characterized by chemotherapy resistance. One of the causes could be chemotherapy-induced senescence. The aim of this study was to assess the expression of senescence pathways in MPM tumor samples taken before and after treatment with cisplatin-pemetrexed. RNA was extracted from 20 MPM tumor samples taken from patients from before and after neo-adjuvant treatment (total of 10 patients).Tumor content was assessed by measuring expression level of mesothelioma markers mesothelin, calretinin and podoplanin relative to histone by real time PCR. The expression of fibroblast activation protein (FAP) was also analyzed. Gene expression was validated by Western blot. A tumor marker expression score was determined and compared to H&E stained slides. Senescence pathways were assessed by quantifying the expression of p21, plasminogen activator inhibitor-1 (PAI-1) for the p21-p53 pathway, IGFbPrP1 for the IGF pathway and ALDH3A for the IFN pathway. A p21-PAI1 and a general senescence score were determined. MPM tumor markers expression in 20 MPM samples demonstrated correlation to tumor to stroma ratio by H&E staining. Three samples with a MPM tumor marker score of less then 10% were excluded from further analysis. FAP expression was detected in all samples. A significant increase in the p21-PAI1 ($p=0.047$) and general senescence ($p=0.021$) scores were observed after chemotherapy. The expression of senescence markers, in particular in the p21-p53 pathway is increased after treatment with cisplatin-pemetrexed.

Collaborations:

- Department of Oncology (Emanuela Felley-Bosco, Claudia Frey, Roy Sidi, Rolf Stahel)
- Department of Clinical Pathology (Alex Soltermann, Holger Moch, Peter Vogt)
- Institute for Biostatistics (Valentin Rousson, Burkart Seifert)
- Department of Radiooncology (Andreas Hollenstein, Martin Pruschy)

Selected references:

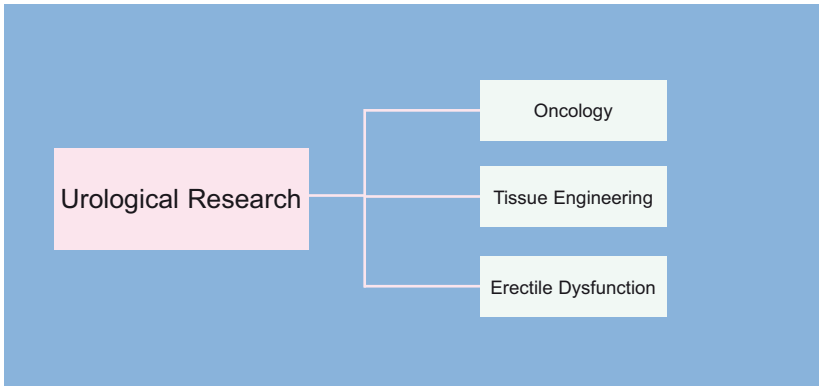
- Thurneysen C, Opitz I, Kurtz S, Weder W, Stahel RA, Felley-Bosco E. Functional inactivation of NF2/merlin in human mesothelioma. Lung Cancer 2008 Oct 3.

2.6 Urological Research

Prof. Dr. med.
Tullio Sulser

Dr. med.
Maurizio Provenzano

Balmer Damina
Scientific Coordinator



2.6.1 Oncology

Dr. med.
Thomas Hermanns

MSc
Giovanni Sais

Dr. med.
Hans-Helge Seifert

Dr. med.
Maurizio Provenzano

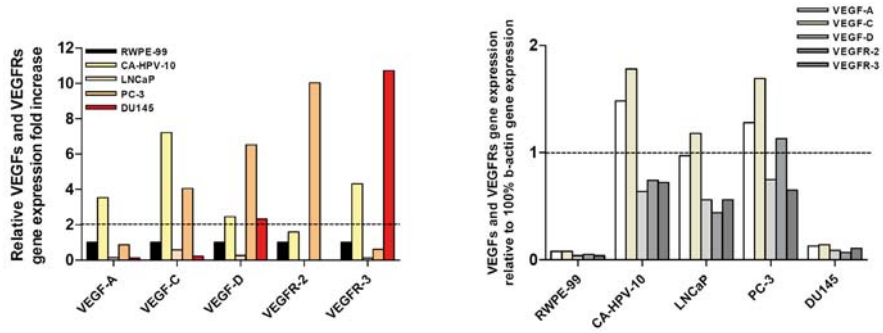
Irina Banzola
PhD

The prognostic value for correlations between lymph-angiogenesis, cancer metastasis and tumor staging in both prostate and bladder cancer patients.
Dr. med T. Hermanns, MSc G. Sais, PhD student, PD Dr. med. H.H. Seifert, Dr. med. M. Provenzano, PhD

A number of angiogenic factors have been indicated to play a role in the promotion of tumor spread in cancer patients. In particular, expression of vascular endothelial growth factors (VEGFs) and their receptors (VEGFRs) in urogenital tract cancers has been suggested to determine a state of cancer invasion and dissemination. In many human cancers, the production of specific VEGFs by tumor cells and, eventually, by chemo-attracted macrophages, leading to an overwhelming lymphatic vasculature within lymph-nodes, has been thus proposed as a target for a future tumor cell dissemination in early cancer stages. In urogenital cancers, VEGF-C and –D produced by both tumor cells and infiltrating macrophages, have been implicated in tumor lymph-angiogenesis and lymph-nodes metastasis through the activation of VEGFR-3 signalling pathways in lymphatic endothelial cells (LECs) and the expression of VEGF-C and VEGF-D has been strongly correlated to lymph-node metastasis in different retrospective studies. Recently, new investigations support also the hypothesis that lymph-angiogenesis could be initiated by direct targeting due to VEGF-C and/or -D and VEGFR-2 interactions. More recently, VEGF-A, a traditional blood vessel-specific growth factor, has been hypothesized to promote tumor lymph-angiogenesis and lymph-node metastasis through VEGFR-2 activation, thus representing the major signalling pathway in lymph-angiogenesis. However, the expression of these factors has not been comparatively evaluated in all different tumor grades and stages and their potential prognostic significance has not been fully explored. Tacking advantage of human cell lines generated from prostate cancer, bladder cancer or normal epithelial and/or urothelial cells, we are aiming at defining the effect of VEGFs produced by tumor cells on endothelial growth, differentiation, migration and tubule formation in urogenital cancers. Our preliminary data suggests the hypothesis that two VEGF factors (VEGF-A,-C) are equally expressed by

prostate cancer cell line tested, as compared to normal epithelial cells, although at different level. Concomitantly, a lower extent of VEGF-D and VEGF receptors 2 and 3 are also expressed (Figure 1). How it might influence tumor growth and spreading has to be better analysed.

Figure 1: VEGFs and VEGFRs gene expression in one normal epithelial cell line (RWPE-99) and four PCa cell lines (CA-HPV-10, LNCaP, PC-3 and DU145)



Collaborations:

- Molecular Tumour Pathology, Department for Surgical Pathology, University Hospital of Zürich.
- Institute for Surgical Research and Hospital Management, Cell and gene therapy section, University Hospital of Basel.

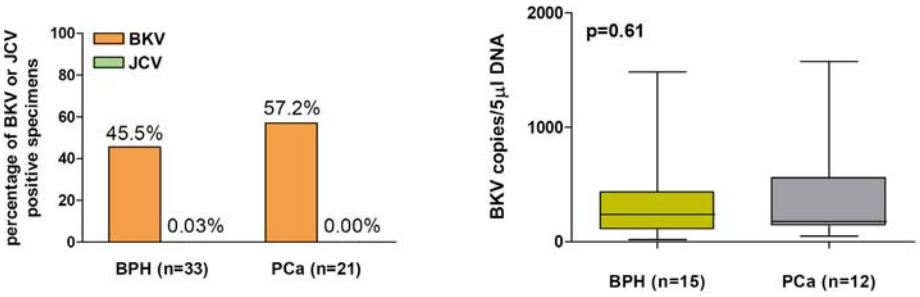
Human polyomavirus BK and genitourinary tract malignancies: BKV LTag immune surveillance in organ specific tumorigenesis and subsequent neoplastic progression.

MSc G. Sais, PhD student, Dr. Irina Banzola, PhD, Dr. med. M. Provenzano, PhD

Prostate cancer (PCa) is a leading cause of cancer death in men. Nearly one third of annually new diagnosed cancers are prostate tumors. A contemporary model for prostate cancer induction and progression should include the potential contribution of inflammation, such as proliferative inflammatory atrophy (PIA), to the development of preneoplastic or neoplastic lesions. Human Polyomavirus BK (BKV) has been associated to pre-early stages of cancer in the urinary tract and it is postulated to play an important role in the pathogenesis of PCa. BKV oncogenisis is due to the ability of the main regulatory protein Large Tumor antigen (LTag) to regulate critical pathways of human cell cycle when BKV infects non permissive cells. In prostate, cytoplasmic colocalization of BKV LTag and p53 has been detected in precancerous lesions. Owing to LTag expression in infected cells, the antigen has been identified as an important target for immune surveillance. It thus prompted us testing whether an inefficient immune response against LTag-p53 binding regions may define a role for BKV LTag immune surveillance in prostate specific tumorigenesis and subsequent PCa progression.

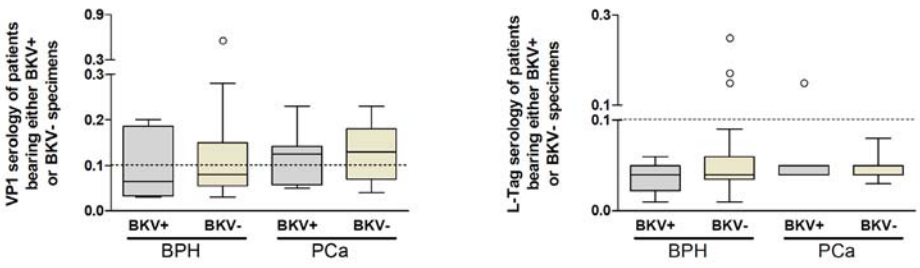
Our previous results suggest that specific BKV LTag peptides nesting within regions responsible for LTag binding to p53 could efficiently be used to test the immune response in BKV seropositive donors (Provenzano et al. 2006). In this study we propose a comprehensive characterization of epitope specific T cell response against BKV LTag in BKV-experienced patients bearing either PCa or benign prostate hyperplasia (BPH), as compared to gender-matched healthy donors. 82 male patients (39 BPH and 43 PCa) and 10 healthy gender-matched donors were enrolled. 45.5% (n=15/33) of BPH and 57.2% (n=12/21) of PCa surgically excised specimens were positive for BKV-LTag DNA detection.

Figure 1: BKV and JCV LTag DNA detection in either BPH or PCa specimens.



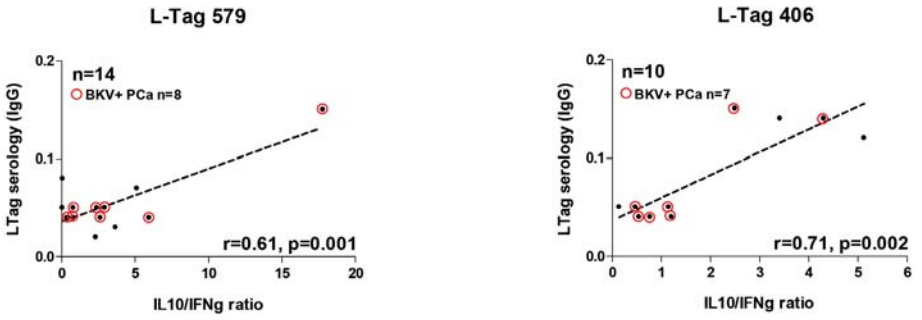
At serological level, 29/33 BPH (89%) and 38/41 PCa (92%) patients clustered in the lower IgG titre range for LTag serology (IgG OD >0.04<0.1). Thus, stratifying our cohorts of patients based on both BKV-LTag DNA detection and IgG serology to LTag, all BPH (9/9, 100%) and nearly all PCa (9/10, 90%) BKV-LTag DNA+ patients clustered within the lowest IgG titre range for LTag serology.

Figure 2: No reactive humoral immune response to L-Tag in PCa and BPH patients bearing BKV+ specimens



In order to test our hypothesis, PBMCs from 15 HLA-A*0201 BPH and PCa patients were ex vivo stimulated with the two immunogenic HLA-A*0201 restricted peptides nesting within LTag-p53 binding domains ($LTag_{406}$ and $LTag_{579}$) (Provenzano et al. 2006). Evidence of a proinflammatory and lytic activity impairment in favour to an immune regulatory function was better observed in PCa than BPH patients by plotting cytokine gene expression (IL-10/IFN-γ) against LTag IgG serology upon both $LTag_{406}$ (r=0.71, p=0.002) and $LTag_{579}$ (r=0.61, p=0.001) peptides induction. Among PCa, 33.4% of BKV-LTag DNA+ patients upon $LTag_{406}$ and 26.7% upon $LTag_{579}$ peptides induction showed evidence of immune regulatory activity and higher Gleason score (≤7). More relevantly, patients showing an increase of CD8+/CD25+/FoxP3+ cells upon both $LTag_{406}$ and $LTag_{579}$ peptides stimulation were prevalently those with both BKV+ specimens and an immune regulatory profiling.

Figure 3: Correlation between cytokine profiling in HLA-A*0201 PCa upon LTag peptides induction and BKV serology, BKV molecular testing and Gleason score.



L-Tag DNA detection	L-Tag serology	Cytokine pattern	number of patients	Gleason score
+	0.15	Th2	1 (7%)	7
+	0.05	Th2		7
+	0.04	Th2		8
+	0.04	Th2	3 (21%)	9
+	0.05	Th1		7
+	0.05	Th1		7
+	0.04	Th1		6
+	0.04	Th1	4 (29%)	7
8 (57%)				
-	0.15	Th2		7
-	0.12	Th2		6
-	0.14	Th2	3 (21%)	6
-	0.03	Th2		7
-	0.03	Th2	2 (15%)	6
-	0.05	Th1	1 (7%)	5
6 (43%)				
			14 (100%)	

L-Tag DNA detection	L-Tag serology	Cytokine pattern	number of patients	Gleason score
+	0.15	Th2	1 (9%)	7
+	0.05	Th2		7
+	0.04	Th2		8
+	0.05	Th2		7
+	0.04	Th2	4 (37%)	9
+	0.05	Th1		7
+	0.04	Th1	2 (18%)	6
7 (63%)				
-	0.12	Th2		6
-	0.14	Th2		6
-	0.03	Th2	3 (27%)	6
-	0.05	Th1	1 (9%)	5
6 (37%)				
			11 (100%)	

Achievements 2008

- SNF grant: Generation of a Recombinant Vaccinia Virus encoding immunogenic BKV Large T antigen/p53 binding domains epitopes to promote the expansion of effector T lymphocytes across a wide range of MHC class I and II antigens in prostate cancer patients
- Provenzano M: The two large T antigen (LTag)-p53 binding domains exert an epitopespecific immune regulatory profile in polyomavirus BK seropositive prostate cancer patients. 2nd International Symposium on Viral Oncology, ICVOR, Philadelphia, PA, September 26-27, 2008 http://www.icvor.org/icvor_meeting_agenda.pdf
- Feder-Mengus Ch, Wyler S et at. Expression of IDO immunosuppressive factor gene and malignant transformation in prostate cancer. 23rd Annual Congress of the European Association of Urology (EAU), Milan, Italy. European Urology Supplements, Volume 7, Issue 3, Pages 275-275
- Provenzano M, Feder-Mengus Ch et al. Indoleamine 2,3-dioxygenase (IDO) expression and malignant transformation in prostate cancer. 2008 ASCO Annual Meeting, Chicago, IL. J Clin Oncol 26: 2008 abstr 5149

Collaborations:

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

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IDO expression and malignant transformation in prostate cancer

Dr. Irina Banzola, PhD, MSc G. Sais, PhD student, Dr. med. M Provenzano, PhD

A number of immunosuppressive factors have been suggested to play a role in the functional impairment of the immune system in prostate cancer (PCa) patients. Among them, indoleamine 2,3-dioxygenase (IDO) has been considered to favour tumoral immune escape based on tryptophan degradation. We recently evaluated the expression of IDO-1 in prostate cancer (PCa) and benign prostate hyperplasia (BPH) tissues from seventy-six patients (34 PCa and 42 BPH). The results from PCa patients showed the existence of two significantly different groups: one with lower levels of IDO-1 gene expression (IDO^{low}) (comparable to those of the BPH patients), and one with higher level of IDO-1 gene expression (IDO^{high}; $p<0.001$). Within IDO^{low} group of patients, immunohistochemistry (IHC) analysis showed expression of IDO-1 prevalently in the endothelial cells of tumor micro environment vessels, whereas in the latter case (IDO^{high}) the presence of IDO-1 was detected exclusively in tumour cells. Furthermore, in the IDO^{high} expressing patients, a significant correlation (R^2= 0,84; p=0,0045) between the level of expression of the gene in tumor specimens and its activity (kynurenine/tryptophan ratio) in related patients' sera was found. Data were also supported by the relevant trend (R^2= 0,58; p=0.13) observed in relation to PCa patients' clinical features (Gleason score) (Feder-Mengus, Wyler et at. 2008)

Testing several normal human tissue and prostate cancer cell lines, we identified different level of expression of IDO-1, although we were able to confirm the absence of its expression in normal prostate. To note is the highest expression of IDO-1 in mature dendritic cells (mDCs; ΔCt=9.93) compared to placenta (ΔCt=15.62) and, more interestingly, the evident decrease in gene expression of IDO-1 in either prostate cancer bone metastases (PC-3; ΔCt =22.59), brain metastases (DU-145; ΔCt=26.68) or lymph-node metastases (LNCaP; ΔCt=26.44), as compared to prostate cancer localized tumor (CA-HPV-10; ΔCt =18.5). No IDO-1 gene expression in normal epithelial prostate cells (NEPC) from a non-bearing tumor subject was detected (Table 1).

Table 1: IDO-1 gene expression in normal human tissues and prostate cancer cells.

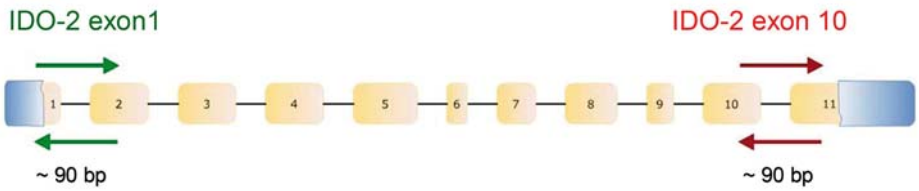
Cell/tissue	*immature DCs*	*mature DCs*	*placenta*	*ovary*	*liver*	*brain*	*prostate*
Delta Ct (ΔCt)	20.53	9.93	15.62	18.14	22.97	29.58	-

PCa cell-lines	*RWPE2-99*	*CA-HPV-10*	*LNCaP*	*PC-3*	*DU-145*	*NEPC*
Delta Ct (ΔCt)	25.09	18.5	26.44	22.59	26.68	-

Data are represented as Delta Ct (ΔCt) relative to the control gene RNA18S (therefore, the higher the ΔCt, the lower the quantity of IDO-1 gene detected).

In order to perform a quantitative analysis of the expression of IDO-2, we have designed two TaqMan assays, one on the region between exon 1 and 2, and the second between exon 10 and 11, spanning exon-intron junctions to prevent amplification of genomic DNA (Figure 1). We have thus defined a protocol for the extraction of total RNA from fresh and paraffin embedded tissues as well as cell lines to be used for the quantitative analysis of IDO-1 and -2 gene's expression in order to compare the levels of constitutive and inducible expression of both isoforms in healthy tissues and prostate cancer cell-lines.

Figure 1: IDO-2 exon 1 and exon 10 assay



Our results confirm those from tissues previously tested by Metz et al. However, our assays provide additional information. The tissue that constitutively expresses IDO-2 at higher levels is the placenta followed by mature DCs, liver and brain. Most interestingly, it appears that in those tissues in which both assays are detected, they are expressed at different levels. In placenta, a ΔΔCt of 5,7(~50 fold difference) between the expression of the exon 10 and exon 1 assays was found. It was even higher in brain (ΔΔCt of 8.1) and in mature DCs (ΔΔCt of 8.6; ~400 folds). The levels of detection of the exon 1 assay represent the levels of expression of the long isoform of IDO-2, whereas the short isoform corresponds to the quantity of the exon 10 assay that exceeds the exon 1 assay. Since the difference between the two assays is so marked (ranging from 50 to 400 folds) we can approximate the detection of the exon 10 assay with the levels of the short isoform. Therefore, we can state that in normal tissue there is a difference in the expression of the short and long isoforms, when both expressed (Table 2). Remarkably, when testing IDO-2 expression levels in the prostate cell-lines, we detected the long isoform only in the localized primary tumour cell-line (CA-HPV-10), thus suggesting a different pattern of expression of the two isoforms in metastasis compared to localized tumour cell-lines. Interestingly is the confirmation of ΔΔCt of 8.6 (400 fold difference) between exon 10 and exon 1 in CA-HPV-10 as well. These preliminary data will have to be confirmed by further investigation on the effect of stimuli upon the pattern of expression in the cell-lines and by the analysis of samples from patients.

Table 2: IDO-2 exon 10 and exon 1 assay in normal human tissues and prostate cancer cells

Cell/tissue	*immature DCs*	*mature DCs*	*placenta*	*ovary*	*liver*	*brain*	*prostate*
Delta Ct (ΔCt)	20.53	9.93	15.62	18.14	22.97	29.58	-

PCa cell-lines	*RWPE2-99*	*CA-HPV-10*	*LNCaP*	*PC-3*	*DU-145*	*NEPC*
Delta Ct (ΔCt)	25.09	18.5	26.44	22.59	26.68	-

Data are represented as Delta Ct (ΔCt) relative to the control gene 18S (therefore the higher the ΔCt, the lower the quantity of IDO-1 gene detected).

From our first analysis it is not possible to evince how many exons are missing in the short isoform of IDO-2 besides exon 1, but since the heme-binding site of the protein is coded by exon 11, we can hypothesize that the short isoform of IDO-2 might be able to code for a functional protein, although no certain data are available about the differences between the two isoforms.

Collaborations:

- Molecular Tumour Pathology, Department for Surgical Pathology, University Hospital of Zürich.
- Institute for Surgical Research and Hospital Management, Oncology section, University Hospital of Basel.
- Department of Clinical Pharmacology, University of Florence, Italy.

Selected references:

- Feder-Mengus C, Wyler S, Hudolin T, Ruszat R, Bubendorf L, Chiarugi A, Weber W, Bachmann A, Gasser T, Sulser T, Heberer M, Spagnoli GC, Provenzano M: Expression of IDO immunosuppressive factor gene and malignant transformation in prostate cancer.
  Eur J Cancer 2008; 44(15): 2266-75.

2.6.2 Tissue Engineering for Urologic Tissues

Dr. med.
Lukas Hefermehl

Dr. med.
Meline Stölting

Dr. med.
Fahd Azzabi

Dr. med.,PhD
Daniel Eberli

Bioengineered Muscle for Functional Sphincter Reconstruction
L.J. Hefermehl, M. Stölting, F. Azzabi, D. Eberli

Approximately one third of women will experience urinary incontinence, the involuntary leakage of urine. Incontinence may be caused by sphincter muscle and/or surrounding tissue damage due to the presence of various conditions, such as those caused by congenital anomaly, trauma, surgery and child birth. Multiple treatment modalities, including surgery and injection therapies into the urinary sphincter region have been tried to restore anatomical structure of the sphincter region with various results. However, none of these methods is able to restore normal sphincter muscle function.
Cell-based approaches to repair damaged tissue function have been proposed and applied experimentally and clinically in a variety of tissues and organs.

The goal of this research is to show that cell therapy can be applied clinically in patients with urinary incontinence. Therefore, the main objectives are to investigate the applicability of the cell-based system for the restoration of sphincter tissue function in a clinical setting. Refinement of the cell culture system that would allow for immediate clinical translation are needed. Special efforts are made to grow cells from all ages. This is of importance since most of the patients suffering from urinary incontinence are over the age of 50.

We were recently able to demonstrate that functional muscle tissue can be engineered using autologous muscle precursor cells (MPC) and that the restoration of sphincter function can be achieved in a canine sphincter insufficiency model as a pre-clinical translational study. Further, we have demonstrated the feasibility of using human muscle precursor cells for clinical application. Human MPCs were expanded using methods compliant with regulatory agencies and characterized using standard techniques. Furthermore, this research was able to show that functional muscle can be engineered from biopsies of all donor ages.
Cellular therapy for sphincter muscle regeneration may provide a definitive treatment modality in patients suffering from urinary incontinence, as well as from other pathologic conditions involving sphincter insufficiency.

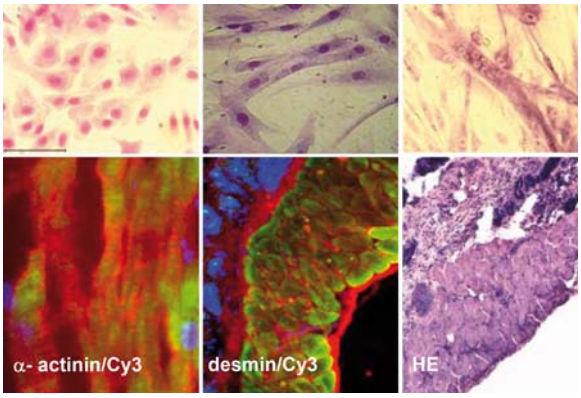


Figure 1
MPCs *in vitro* (top row) and Muscle formation *in vivo* (bottom row) after MPC injection.
Top: MPC in culture, cell fusion and myofiber formation at different time points (Giemsa staining). Bottom: muscle tissue *in vivo* after dorsal subcutaneous injection in nude mice (left to right: α-actinin/Cy3, desmin/Cy3 and HE).

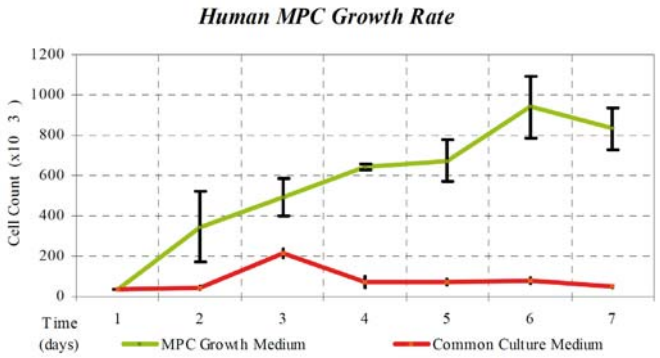


Figure 2
Growth optimization of human muscle precursor cells using defined media.

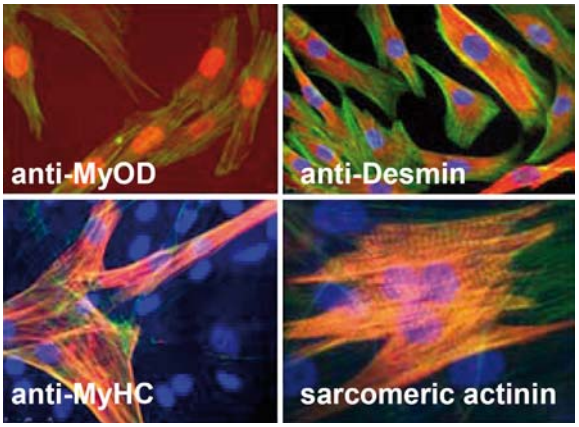


Figure 3
MPC characterization by Immunohistochemistry (anti-MyOD, anti-Desmin, anti-MyHC and anti-sarcomeric actinin).

Collaborations:

- Department of Urology and Wake Forest Institute for Regenerative Medicine, Wake Forest University School of Medicine, Winston-Salem, NC, USA

2.6.3 Erectile Dysfunction

Dr. med.
Alexander Müller

Penile Rehabilitation after Radical Prostatectomy:
The cavernous nerve crush injury model in the rat
Dr. med. Alexander Müller

A. Radical Prostatectomy and Erectile Dysfunction

Erectile dysfunction (ED) is a recognized sequela of radical prostatectomy (RP). Despite advances in nerve spring techniques, the operation is associated with a significant incidence of ED which occurs between 30-80% depending on the literature. The mechanisms proposed include neuropraxia-induced structural damage to erectile tissue, chronic erectile absence associated structural damage and vascular alterations. Over the past 10 years there has been a recrudescence in interest in clinical and basic scientific research in post-radical prostatectomy erectile dysfunction. It has been suggested that the use of pharmacologic agents, including phospodiesterase inhibitor type 5, in the early stages after RP can result in improved erectile function outcomes. Reducing the incidence of this problem after RP will lead to significant improvement in quality of life of such patients and will likely result in cost savings to health care systems because of the reduced need for long-term erectogenic medications or surgery for drug-refractory ED.

B. The Concept Of Penile Rehabilitation

The relationship between hypoxia and cavernosal fibrosis has been documented in several in vitro studies. It has been shown that cavernosal smooth muscle cells exposed to hypoxia underwent an increased collagenization. Since hypoxia of cavernous tissue is related to the blood supply and the greatest blood supply occur at time of erection any neural damage that results in ED may expose the cavernous tissues to longer periods of hypoxia and consequently structural damage. The current literature provides evidence that events of nocturnal erection oxygenate the cavernosal tissue (concept of cavernosal oxygenation), and this might protect them from developing fibrotic changes during the transient period of erectile dysfunction following nerve sparing radical prostatectomy.

C. Cavernous Nerve Injury Model in Rats

Quinlan et al in 1989 first described the rat model of CN injury for the study of RP-associated erectile function changes. Further evolution of this model let to the world wide acceptance of this model to reliable assess functional and structural sequalae of neural trauma in the corporal tissue of the rat penis after CN injury. The assessment of erectile hemodynamics in the rat model has matured enough to allow objective assessment of the functional parameter reporting the ICP/MAP ratio between the maximum intracavernosal pressure (ICP) and the corresponding mean arterial blood pressure (MAP) measured during electrical stimulation of the CN. The reports on the neuroprotective and neuroregenerative qualities of pharmacologic agents and interest in exploring other potentially neuromodulatory strategies have increased the interest of this reproducible rat CN injury model, that has extrapolatability to the human.

This model seems to be representative of neural injury that occurs at the time of pelvic surgery and thus, allow the assessment of the neuromodulatory

properties of pharmacologic strategies in a pre-clinical fashion prior to human clinical trials.

D. Preliminary Studies

In preliminary studies the principle investigator was able to demonstrate that the functional and structural consequences of bilateral CN injury were ameliorated by the daily use of the PDE5i sildenafil citrate. After bilateral CN crush injury applied in mature Sprague-Dawley rats the erectile function (ICP/MAP ratio) improved with sildenafil in a time a dose dependent fashion with maximization of erectile function recovery occurring with daily 20mg/kg sc at the 28 day time-point and resulted in smooth muscle-collagen ratio protection and CD31 and eNOS expression preservation (Figure 3 and 4). Furthermore sildenafil increased phosphorylation of AKT and eNOS and reduced intracavernosal apoptosis (Figure 5).

Supporting the above mentioned cavernosal oxygenation concept as a protective mechanism for erectile function we were able to document improved erectile function preservation after hyperbaric oxygen therapy in the cavernous crush injury model in rats. The effects appeared to be mediated via preservation of neurotrophic and endothelial factor expression.

Also with the use of the immunophilin ligand FK506 ascertaining an optimal dose and timing of the drug we were able to show that short-term treatment with doses of FK506 sc higher than previously utilized preserves erectile function in the rat CN injury model. Pre-treatment did not offer an advantage but FK506 administration just prior to CN injury and for a short time post-injury achieved the best functional outcomes. The benefits of this pharmacotherapeutic strategy appeared to be mediated through reduction in cavernosal apoptosis and of nerve injury-associated perturbations in neurotrophic factor expression which might be the reason for a dramatic structural preservation seen under transmission electron microscope in the treatment animals compared to control. Based on promising animal experimental data in this CN crush injury model the future role of FK506 as a pharmacologic neuromodulator in the RP population will be defined by the results of randomized, placebo-controlled trials, which are ongoing.

With this upcoming year 2008 we would like to establish the aforementioned cavernous nerve crush injury model as part of the Urological Laboratory at the USZ to continue this part of promising research aiming for helpful strategies in penile rehabilitation after radical prostatectomy which can be brought from bench to bed side.

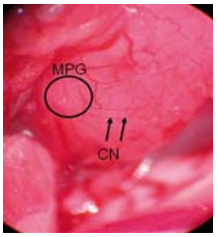
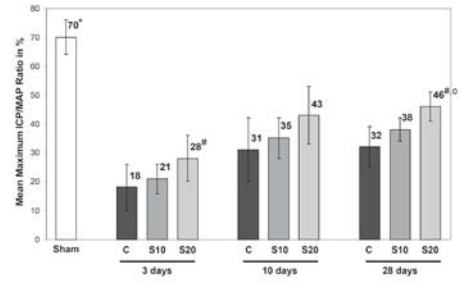


Figure 1:
Cavernous Nerve in the Rat Intra-operative picture showing the cavernous nerve (CN) coming from the major pelvic ganglion (MPG) and running along the surface of the prostate in the rat.

Figure 2:
Intra-operative picture displaying the cavernous nerve (CN) hooked up by an electrode for electrical stimulation to measure the maximum intracavernosal pressure (ICP). At the same time of CN simulation the corresponding mean arterial blood pressure (MAP) will be reported as the ICP/MAP ratio representing a parameter of erectile function.



* significantly higher compared to all other groups (p<0.001),
# significantly improved compared to corresponding C group (p<0.05),
o significantly improved compared to S10 at 28 days (p=0.01).

Figure 3: Functional Results
Graph showing the mean maximum intracavernosal pressure (ICP) divided by the corresponding mean arterial pressure (MAP), reported as ICP/MAP ratio as a percentage for Control (bilateral CN crush), and both treatment groups S10 (daily 10 mg/kg sildenafil sc) and S20 (daily 20 mg/kg sildenafil sc) at different time points (3, 10, and 28 days).

* significantly higher compared to all other groups (p<0.001),
\# significantly improved compared to corresponding C group (p<0.05),
o significantly improved compared to S10 at 28 days (p=0.01).

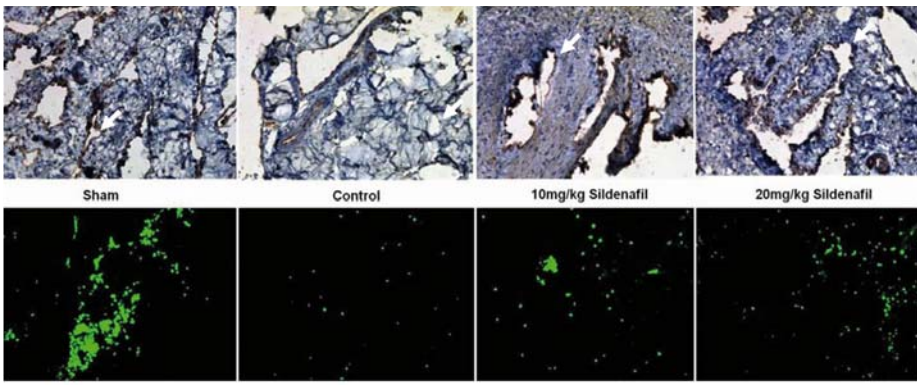


Figure 4: Immunohistochemistry staining for CD31
At 28 days after cavernous nerve injury the Control group (bilateral CN crush) demonstrated a lower density of CD31 staining compared to Sham (no CN crush) and both treatment groups S10 und S20 displayed higher staining compared to control on both immunohistochemistry (upper panel) and immunofluorescence (lower panel).

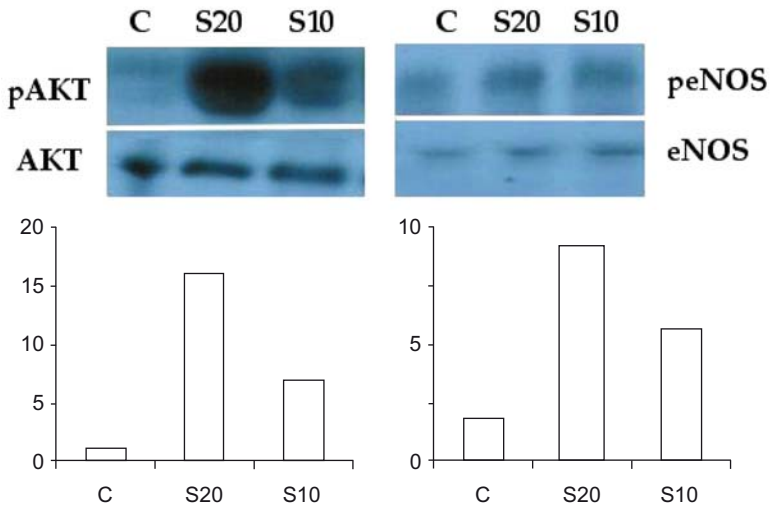


Figure 5: Immunoblotting for eNOS/AKT
Both treatment groups S10 and S20 (10 and 20 mg/kg sildenafil sc daily) demonstrated greater activation (phosphorylation) of AKT and eNOS compared to the Control group C.

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  23rd Annual EAU Congress, 26-29 March 2008, Milan, Italy

Collaborations:

- Prof. J.P. Mulhall, Laboratory of Sexual Medicine, Memorial Sloan-Kettering Cancer Center, New York, NY, USA.
- Dr. Juan Martinez-Salamanca, Department of Urology, University Hospital Madrid, Spain
- PD Dr. R. Graf, Division of Visceral & Transplant Surgery, USZ, Zürich

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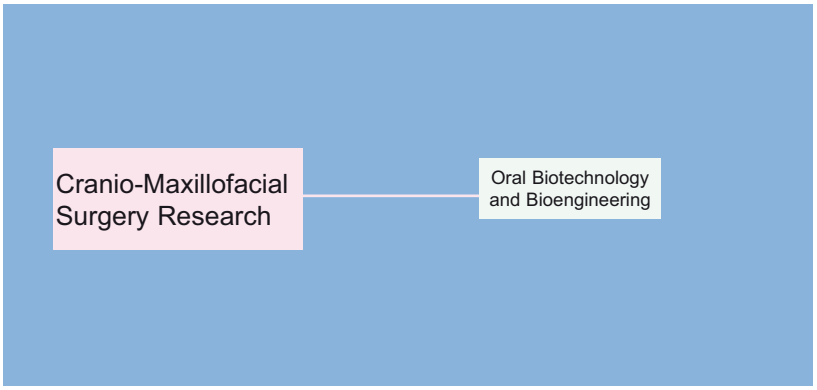
2.7 Cranio-Maxillofacial Surgery Research


Prof. h.c. PD
Dr. rer. nat.
Franz E. Weber


Prof. Dr. Dr
Klaus W.Grätz



2.7.1 **Oral Biotechnology and Bioengineering**


Prof. h.c. PD
Dr. rer. nat.
Franz E. Weber


Martin Ehrbar
PhD


Katrin Lange
PhD


Ana Sala
PhD-student


Rita Correro
PhD-student


Patrick Hänseler
PhD-student


Alexander
Tchouboukov


Yvonne
Bloemhard


Dr. med
Astrid Kruse


Prof. PhD
Heinrich Walt

BMP and bone regeneration
Franz Weber, Martin Ehrbar, Katrin Lange, Ana Sala, Rita Correro, Patrick Hänseler, Alexander Tchouboukov, Yvonne Bloemhard, Astrid Kruse, Heinrich Walt

Growth factor mediated bone regeneration
In Cranio-Maxillofacial Surgery, orthopaedics, and dentistry improved treatments for bone regeneration, and bone augmentation are in need. Valuable tools for this purpose are single growth factors like bone morphogenetic proteins (BMPs), or vascular endothelial growth factors (VEGF), hormones like parathyroid hormone (PTH), mixtures of growth factors and hormones or even autologous mixtures of factors as present in platelet rich plasma. The goal of our research is to identify the best growth factors or mixtures thereof and to develop novel delivery systems aiming towards an optimization for specific applications. Another strategy, recently developed in our laboratory is the use of agents which enhance the biological effect of growth factors, in particular of BMPs. For the future we hope to combine novel delivery systems, different growth factors and hormones, and synergistic agents, which in the end could reduce the risk and the costs for the clinical application of hormones and growth factors for bone repair and bone augmentation.

Synthetic hydrogels
Learning from nature and using those evolutionarily evolved principles is always a very successful strategy for tissue regeneration and tissue engineering. Bioengineering can then use those principles and apply them in conjunction with synthetic materials to generate safe novel biomaterials. The emphasis of our work is the generation of engineered artificial extracellular Matrices (aECM) which can readily be adapted to physical demands and are able to regulate cell behaviour to promote the regeneration or de novo formation of tissues inside the body or from tissue culture. Despite reported progress on such materials is impressive, the bioactivity of state of the art aECM is too reductionistic compared to collagen or fibrin based gels.

At present we are investigating a novel class of Polyethylene Glycol (PEG) based hydrogels that are formed by a transglutaminase enzymatic reaction and can be termed as synthetic fibrin hydrogels. These hydrogels can be engineered by combining different key characteristics for cell maintenance and differentiation, such as cell adhesion ligands, protease sensitive sites or bioactive molecules independent of physical properties to form a suitable sECM for tissue regeneration applications.

Bone substitute materials

Tissue engineering is emerging as a significant potential alternative or complementary strategy whereby tissue and organ failure is addressed by implanting natural, synthetic, or semi-synthetic tissue and organ mimics that are functional from the start or that grow into the required functionality.

1) Cell sheet engineering

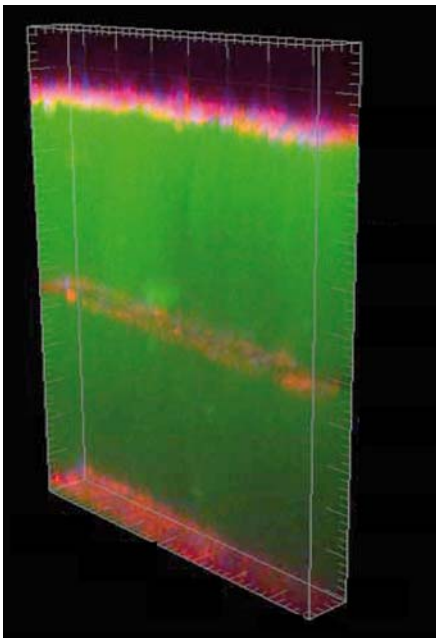
In this project we seek to develop three-dimensionally designed cell-polymer composite materials for tissue engineering. The basis of the project is a new method that allows for the harvesting of cell sheets by electrochemical means. In combination with dedicated micro-patterning techniques this method allows for the control of the spatial organization of cells in two dimensions. We propose to overcome the existing critical problems in cell-sheet engineering by developing a three dimensional composite material consisting of alternating layers of such two dimensionally engineered, heterotypic cell sheets and microstructured biodegradable natural and artificial polymeric thin film hydrogels.

2) Synthetic HA/TCP based bone blocks for vertical bone augmentation

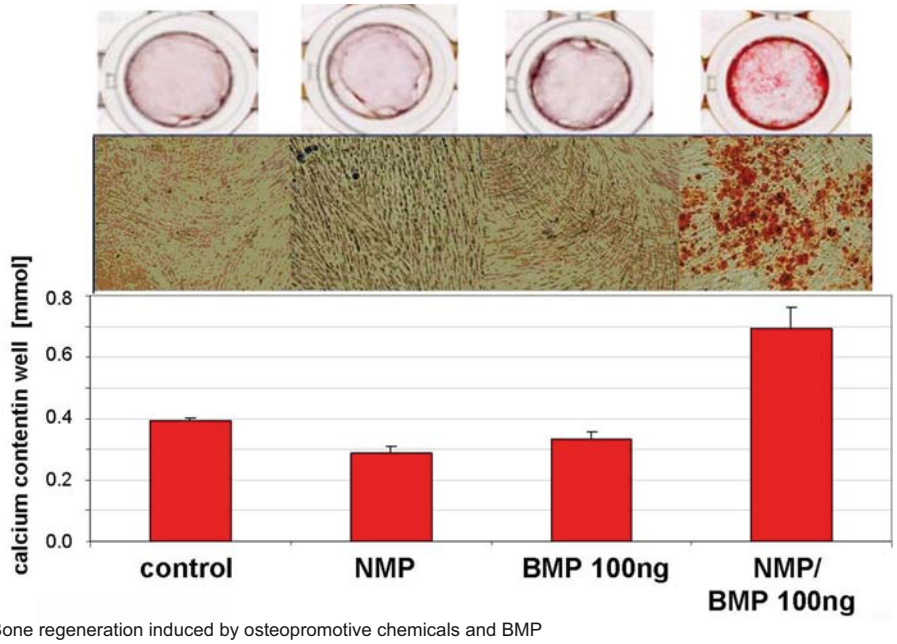
The goal of this subproject is the development of synthetic HA/TCP based with facilitated cell in-growth characteristics and an facilitated differentiation into the osteoblastic lineage. In addition novel strategies to combine growth factors and HA based materials are also evaluated.

3) Porous bioactive glass as bone substitute material

“bioactive” substances are capable of forming a bone-like apatite layer on their surface, similar to our naturally occurring bone hydroxyapatite. Some of the main bioactive ceramics used clinically are: bioactive glass within the Na2O-CaO-SiO2-P2O5 system, hydroxyapatite and sintered -tricalcium phosphate. Out of this group, bioactive glass has been furthermore shown to promote osteoblastic differentiation in vitro, as well as osteoinduction in vivo; therefore, there is a great interest to study its potential in bone regeneration. The main goal of this project is to determine the effect on the differentiation of preosteoblasts to osteoblasts by pre-treating 3D-bioglass scaffolds with simulated body fluid.



3D tissue construction composed of 3 cell sheets separated by two synthetic matrices



Bone regeneration induced by osteopromotive chemicals and BMP

Mechanobiology of cartilage and cartilage tissue engineering

Our objective is to gain knowledge of the mechanobiology of temporomandibular joint (TMJ) cartilage. The first part of this project was dedicated to design and build an apparatus able to mechanically stimulate nasal septum cartilage. At present we apply different stimulation regime to test their effect on the maintenance and degradation of cartilage. The long-term objective of this research is to understand the pathomechanics of TMJ degeneration. A second aspect of this research is cartilage tissue engineering by using this mechanical testing system as mechanical stimulator for tissue engineered cartilage.

Nanomedicine

Despite decades of intense research, progress in cancer therapy is relatively slow. In part, it is hampered by the current lack of appropriate mechanisms to transfer anticancer drugs selectively to tumour tissues, thereby limiting their therapeutic potential. This general problem also applies to the treatment of head and neck Squamous cell carcinoma, a disease group of considerable impact, being the sixth most common neoplasm worldwide. Current clinical intervention strategies include ablative surgery with postoperative chemo-radiotherapy where appropriate. However, the introduction of novel less invasive therapeutic concentration regimes that minimize the exposure of normal tissues while maintaining therapeutic concentration in tumours would be extremely meaningful.

In the current research project, we aim to investigate the potential of novel silica-based core-shell nanoparticles for the therapy of oral cancers. Nanoparticles will serve as delivery vehicles that are chemically designed to carry the established chemotherapeutic drug cisplatin in addition to a photosensitizing agent (5-ALA, mTHPC or hypericin) for improved cancer targeting and killing. Our drug targeting concept is thus based on the novel combination of established drug compounds and on the introduction of new cancer targeting systems.

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Collaborations:

- Department of Fixed and Removable Prothodontics and Dental Material Science, University of Zurich, Switzerland (Prof. Ch. Hämmerle, PD Dr. Ronald Jung, Dr. Daniel Thoma)
- Department of Masticatory Disorders, University of Zurich, Switzerland (Prof. Sandro Palla, Prof. Luigi Gallo)
- Division of Obstetrics (Prof. Roland Zimmermann, PD Dr. Andreas Zisch)
- ETH Zurich, Laboratory of Biosensors and Bioelectronic (Prof. Janos Vörös)
- ETH Zurich, Department of Materials (Prof Marcus Textor, PD Dr. Heike Hall-Bolic)
- ETH Zürich Institut f. Biomechanik (Prof. Ralph Müller)
- EPFL Institute of Bioengineering (Prof. Jeffrey Hubbell, Prof. Matthias Lütolf)
- ETH Zürich, Department of Chemistry and Applied Biosciences (Prof Wendelin Stark)
- Universität Belgrad (Serbien-Montenegro) (Dr. Vladimir Kokovic, Prof. Aleksa Markovic und Prof. Milan Jurisic)
- Universität Hongkong Prof. Lim Cheung und Prof. Roger Zwahlen.
- Kuros Biosurgery (Zurich, Switzerland)
- Straumann AG (Waldenburg, Switzerland)
- Inion OY (Tampere Finland)
- Geistlich AG (Wohlen, Switzerland)
- Artoss AG (Rostock, Germany)
- Z-Systems (Konstanz, Germany)
- Degradable solution (Zurich, Switzerland)

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2.8. Surgical Intensive Care Medicine

PD Dr. med.
John F. Stover

Prof. Dr.
Reto Stocker

Silke Ludwig

Jutta Sommerfeld

Angela Fendel

Improvement of intensive care treatment in patients suffering from severe traumatic brain injury

Search for safe arterial blood glucose level following severe traumatic brain injury

Increased as well as decreased arterial blood glucose concentrations contribute to evolving brain damage following traumatic brain injury by inducing neurological deterioration, impairing neurological recovery, and increasing morbidity and mortality. In clinical routine it is essential to avoid arterial blood glucose levels exceeding 10 mmol/ l. Elevated arterial blood glucose levels are corrected by applying insulin which, however, can also induce hypoglycemia which is also detrimental since hyper- as well as hypoglycaemia induce cellular swelling and brain edema due to energetic disturbance and mitochondrial damage. To investigate which arterial blood glucose levels are associated with the least incidence of complications as e.g., induced hypoglycaemia, signs of cerebral metabolic impairment and to determine the safest lowest arterial blood glucose levels a series of investigations were performed in the following three clinical projects. As a consequence we are now avoiding tight low arterial blood glucose levels and are maintaining arterial blood glucose levels between 6 and 8 mmol/ l.

Clinical projects

1) **Retrospective analysis of different arterial blood glucose targets (3.5- 6.5 vs. 5- 8 mmol/ l) on complications and mortality**
2) **Effect of different arterial blood glucose levels on cerebral metabolism assessed by arterial and jugular venous differences**
3) **Influence of arterial blood glucose levels on cerebral glucose concentrations and metabolic parameters determined by cerebral and subcutaneous microdialysis**

Main results project 1 (figures 1 and 2)
In 228 propensity matched patients (age, sex and injury severity) treated in our intensive care unit (ICU) from 2000 to 2004, we retrospectively evaluated the influence of different predefined blood glucose targets (3.5 to 6.5 versus 5 to 8 mmol/l) on frequency of hypoglycaemic and hyperglycaemic episodes, insulin and norepinephrine requirement, changes in intracranial pressure and cerebral perfusion pressure, mortality and length of stay on the ICU. Mortality and length of ICU stay were similar in both blood glucose target groups. Hypo- as well as hyperglycaemic values were significantly increased in the 3.5 to 6.5 mmol/l group, predominantly during the first week. Insulin and norepinephrine requirements were markedly increased in this group. During the second week, the incidences of intracranial pressure exceeding 20 mmHg and infectious complications were significantly decreased in the 3.5 to 6.5 mmol/l group.
Conclusions: Maintaining blood glucose within 5 to 8 mmol/l appears to yield greater benefit during the first week. During the second week, 3.5 to 6.5 mmol/l is associated with beneficial effects in terms of reduced intracranial hypertension and decreased rate of pneumonia, bacteraemia and urinary tract infections.

It remains to be determined whether patients might profit from temporally adapted blood glucose limits, inducing lower values during the second week, and whether concomitant glucose infusion to prevent hypoglycaemia is safe in patients with post-traumatic oedema.

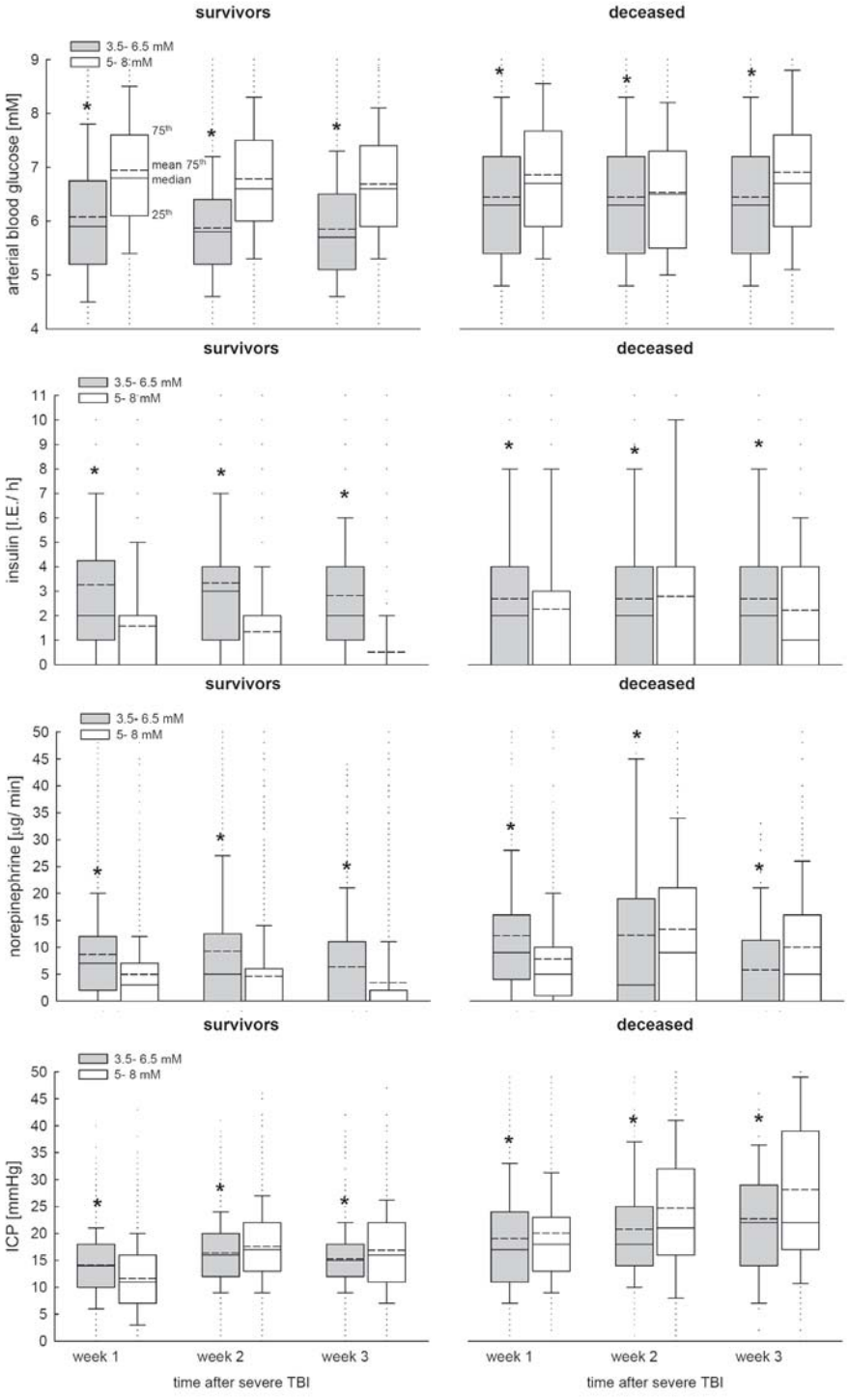

Figure 1
Changes in arterial blood glucose, insulin and norepinephrine dose, and intracranial pressure (ICP) in surviving patients and in those who died within the two different blood glucose target groups (3.5- 6.5 vs 5- 8 mmol/ l) over time.

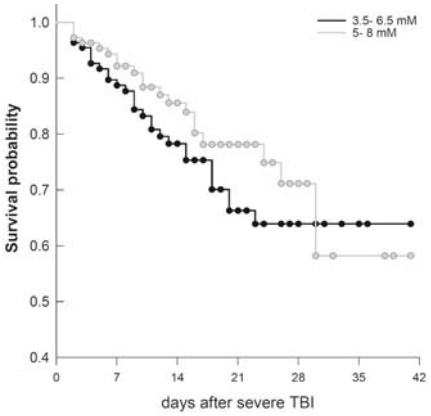

Figure 2
The Kaplan-Meier survival curve illustrates a trend toward increased mortality during the first 2 weeks in patients subjected to blood glucose target 3.5- 6.5 compared to 5- 8 mmol/ l.

Main results project 2 (figure 3)
In 69 patients arterial blood glucose significantly influenced signs of cerebral metabolism reflected by increased cerebral glucose uptake, decreased cerebral lactate production, reduced oxygen consumption, negative LGI and decreased cerebral CO2/HCO3 production at arterial blood glucose levels above 6 to 7 mmol/l compared with lower arterial blood glucose concentrations. At blood glucose levels more than 8 mmol/l signs of increased anaerobic glycolysis (OGI less than 6) supervened.
Conclusions: Maintaining arterial blood glucose levels between 6 and 8 mmol/ l appears superior compared with lower and higher blood glucose concentrations in terms of stabilised cerebral metabolism. It appears that arterial blood glucose values below 6 and above 8 mmol/l should be avoided. Prospective analysis is required to determine the optimal arterial blood glucose target in patients suffering from severe TBI.

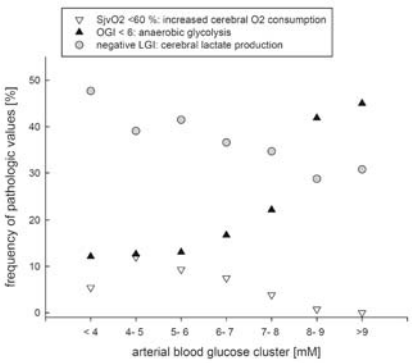

Figure 3
Calculation of frequency of pathological values within pre-defined arterial blood glucose clusters. Values are given for increased cerebral oxygen consumption [jugular venous oxygen saturation (SjvO2) less than 60%), sustained anaerobic glycolysis (oxygen-glucose index (OGI) less than 6), and increased cerebral lactate production (negative lactate-glucose index (LGI)]. With elevated arterial blood glucose the rate of increased cerebral oxygen consumption (SjvO2 less than 60%) was reduced which coincided with decreased rate of increased cerebral lactate production (negative LGI). However, frequency of anaerobic glycolysis (OGI less than 6) was increased at arterial blood glucose levels exceeding 8- 9 mmol/ l.

Main results project 3 (figure 4)
Retrospective analysis of cerebral and subcutaneous glucose concentrations in 12 patients with severe traumatic brain injury revealed that tissue glucose levels were significantly influenced by arterial blood glucose levels between 4 and 8 mmol/ l. A further increase in arterial blood glucose coincided with a plateau within the brain and subcutaneous tissue. This precluded the necessity of increasing arterial blood glucose concentrations above 8 mmol/ l which has been shown to induce metabolic impairment as judged by calculated arterial and jugularvenous differences in metabolic parameters (see figure 3).

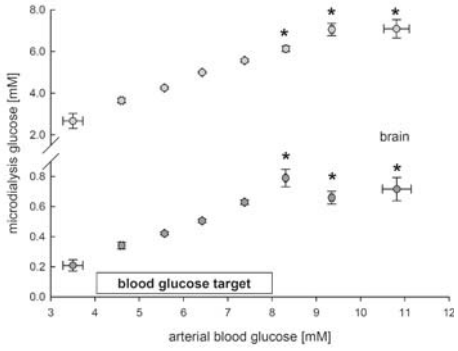

Figure 4
Two- dimensional plot depicting changes in subcutaneous (grey circles) and cerebral (dark grey circles) interstitial glucose determined by microdialysis within pre-defined arterial blood glucose levels in 21 patients suffering from severe traumatic brain injury. Microdialysis glucose levels were significantly elevated by increased arterial blood glucose concentrations with a plateau at arterial blood glucose levels above 8 mmol/ l.

Collaborations:

- Dr. med. Lars Asmis, Institut für Klinische Hämatologie
- PD Dr. Marius Keel, Dr. rer. nat. Luc Härter, Ursula Steckholzer, Klinik für Unfallchirurgie

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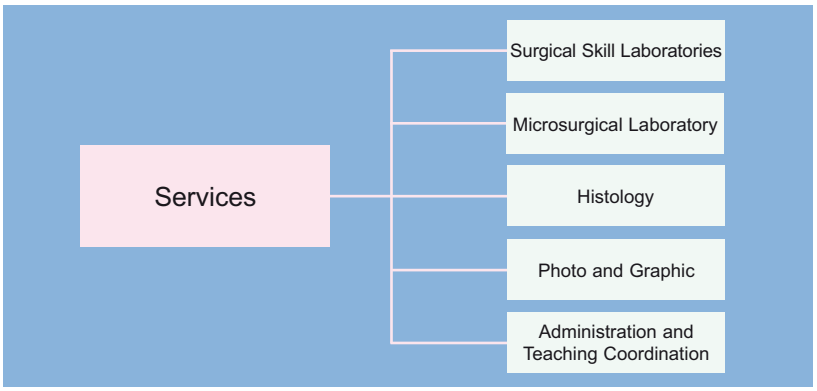
3. Services




Boris Leskosek


Alush Avdyli

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 enviroment. To perform operations under conditions similar to the clinical situation, technical help is provided by our staff which is also responsible for maintenance of our facilities.


Vlasta Strohmeier

3.2 Microsurgical Laboratory

The microsurgery laboratory is a seperate section in which several operating-microscopes are availabe 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.


Astrid Morger

3.3 Histology

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

3.4 Photo and Graphic Services

Nico Wick,
Photographer

Lea Schütz-Cohen,
Photographer

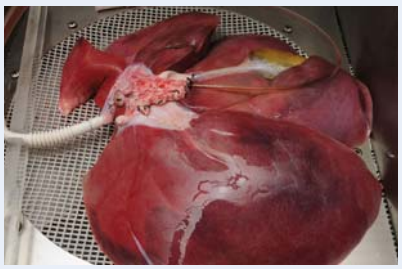
Stefan Schwyter,
Scientific
Illustrator

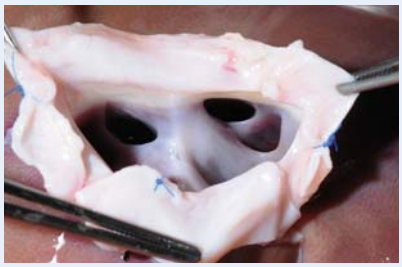
Carol De Simio,
Scientific
Illustrator

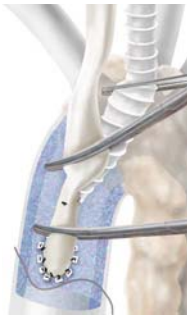
A quick, flexible, versatile and professional service.

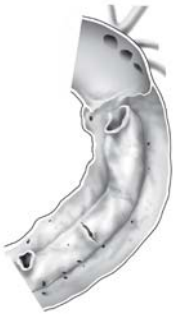
We offer

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

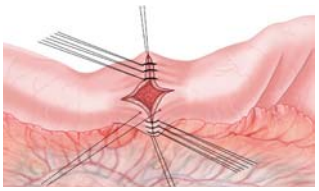


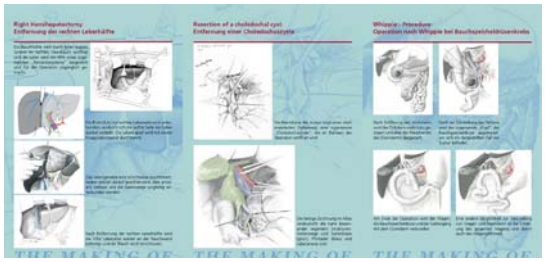


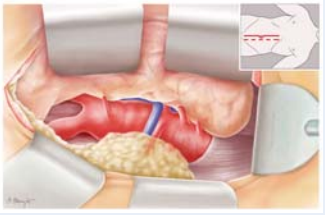




LGID Foundation
Liver and Gastrointestinal Disease Foundation







3. 5 Administration

Susanne Frehner
Administration
Division of Surgical
Research

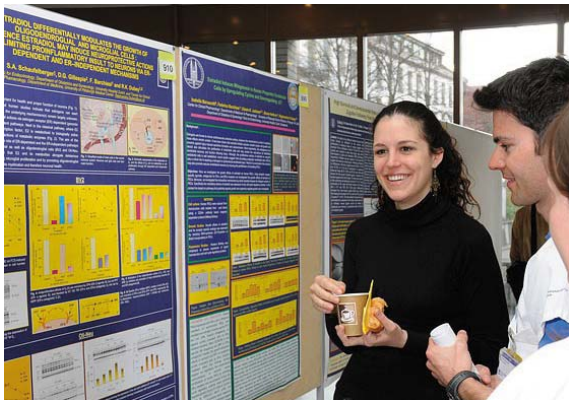
- Administrative office management
- Financial accounting of the Research Divison
- Organisation, planning and coordination of Workshops and vocational training
- Workshop, tutorials and seminars
- Quarterly reports
- Meeting organisation and coordination
- Personnel administration

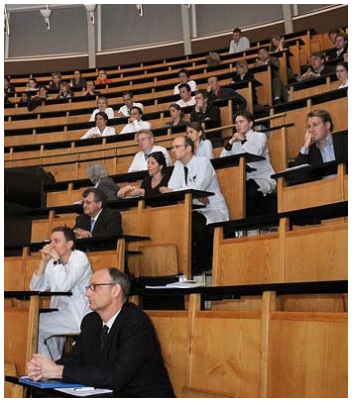
3. 6 Teaching Coordination

Corinne Renold,
Teaching Coordination
Division of Surgical
Research

- 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; excluded are the clinical rotations during the 5th year of study. The work is done in cooperation with the University of Zurich and the University Hospital Zurich for the Department of Surgery.

4. Events and Workshops at the Division of Surgical Research in 2008





8th Day of Clinical Research

Sewing class for medical students

Christmas party

5. Publications 2008

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6. Grants 2008

Cardiac Surgery

Grants	Title of Project	Project Leader
EU Grant Framework Program 6 (BioSys)	Intelligent Biomaterial Systems for Cardiovascular Tissue Repair	Prof. Zünd
Swiss National Science Foundation	Development of Cell-Based Therapies for Myocardial Defects	Prof. Hoerstrup
Hartmann-Müller-Stiftung	Prenatal Human Progenitor Cells for Tissue Engineering of Living Autologous Pediatric Cardiovascular Replacements	Dr. D. Schmidt Prof. Hoerstrup
Novartis Stiftung	Tissue Engineering Heart Valves	Prof. Zünd Prof. Hoerstrup
Bundesministerium für Bildung und Forschung (BMBF Grant)	Cryopreserved Umbilical Cord Cells For Heart Valves Tissue Engineering	Prof. Hoerstrup
Zurich Center for Integrative Human Physiology Grant 2006 – 2008	Vulnerable atherosclerotic plaques - early detection, functional imaging, targeted treatment	Prof. Hoerstrup
National Competence Center of Research	Klinik für Herz- und Gefässchirurgie - Lead Cardiac Robotic Surgery Switzerland	Prof. Zünd
CO-ME	Robotics in Cardiovascular surgery	Prof. Zünd PD Dr. Grünenfelder
Swiss Foundation for Research on Muscle Diseases	"M-band alterations characterize muscle pathogenesis"	R. Schönauer
Wolfermann-Nägeli-Foundation	"The role of M-band in the striated muscle sarcomere"	I. Agarkova
Atricure inc.	Cosgrove Gillinov Clip Studie	S. Salzberg
Roche Research Foundation	Sarcomere remodeling in the failing heart: implications for the disease mechanism	R. Schönauer
Stiftung f. Forschung an der med. Fakultät der Universität Zürich	Sarcomeric M-band as a novel marker for the remodelling process in cardiomyopathy	R. Schönauer

Visceral & Transplant Surgery

Grants	Title of Project	Project Leader
Hepatobiliary laboratory		
SNF	Small-for-size liver transplantation: platelets and platelet-derived serotonin in the ischemic and regenerating liver	Prof. P.A. Clavien
SNF	In vivo analysis of liver regeneration and tissue repair in rodents using an animal MR	Prof. P.A. Clavien
SNF	Hypothermic oxygenated perfusion extracorporeal of the rat liver in non heart beating donors after cold storage	PD Dr. P. Dutkowski
Edoardo R., Giovanni, Giuseppe und Chiarina Sassella-Stiftung	Serotonin Antagonist	Dr. C. Soll/ Prof. P.A. Clavien
Roche Organ Transplantation Research	Protective Mechanisms of Pentoxyfilline for Liver Surgery and Liver Transplantation	PD. Dr. H. Petrowsky/ Prof. P.A. Clavien
Désirée und Niels Yde Stiftung	Liver Cancer	Dr. J.-H. Jang/ Prof. P.A. Clavien
Krebsliga	Pathways in HCC	Dr. C. Soll/ Prof. P.A. Clavien
Pancreatitis laboratory		
SNF	The role of COX-2 in chronic pancreatic inflammation and fibrosis	PD Dr. R. Graf
Velux Stiftung	The role of macrophages in chronic pancreatic inflammation	PD Dr. R. Graf
Amelie Waring Stiftung	Chronische Pankreatitis	PD Dr. R. Graf
Islet-Transplantation laboratory		
UBS-Grant	Der Einfluss des Microenvironments auf die Differenz-ierung von Stammzellen	Dr. P. Kugelmeier
Theodor und Ida Herzog-Egli Stiftung	The impact of the microenvironment on the differentiation of stem cells	Dr. P. Kugelmeier
SNF		Prof. P. Schneider

Trauma Surgery

Grants	Title of Project	Project Leader
SNF	Wound Healing in Vacuum Assisted Closure-Treated Patients after Trauma: Implications of Neutrophil Activation for Accelerated Angiogenesis	Dr. Keel, Dr. Härter, Dr. Labler
AO Research Foundation	Assessment of soft tissue and periosteal micro-circulation in severely open fractures using orthogonal polarozation spectral imaging	Dr. Wanner
Stiftung für wissenschaftliche Forschung der Universität Zürich	Nichterythroide Wirkungen von humanem rekombinaten Erythropoietin in der Traumatologie und rekonstruktiven Chirurgie der Extremitäten	Dr. Wanner

Plastic Hand & Reconstructive Surgery

Grants	Title of Project	Project Leader
SUVA und Jubiläumsstiftung	Tissue Engineering	Dr. Wedler
Swiss Life	Tissue Engineering	Dr. Wedler
Helmut Horten Stiftung	„role of exogenously administred recombinant erythropoietin in plastic surgery“	Dr. C. Contaldo/ Prof. P. Giovanoli

Thoracic Surgery

Grants	Title of Project	Project Leader
Krebsliga	Prognostic markers for malignant pleural mesothelioma	PD Dr. Schmitt-Opitz
Krebsliga Zürich	Establishment of an integrated tumor tissue platform and its application for comprehensive analyses of molecular parameters in lung tumors	Prof. W. Weder
Hartmann-Müller-Stiftung	The effect of NSAIDs on early inflammatory response after mechanical pleurodesis in a pig model	PD Dr. Schmitt-Opitz
Fellowship European Society of Medical Oncology	Intrapleural therapy after surgery for malignant pleural mesothelioma	PD Dr. Schmitt-Opitz
Olga Mayenfisch-Stiftung	Tissue-engineering zur Trachealrekonstruktion	PD Dr. Hillinger
Krebsliga	Epstein Barr virus-induced molecule 1 ligand chemokine in lung cancer therapy	PD Dr. Hillinger
Sassella-Stiftung	Epstein Barr virus-induced molecule 1 ligand chemokine (ELC⁄CCL19)	PD Dr. Hillinger
SNF	Immune targeted therapy for lung cancer	PD Dr. Hillinger
EMDO-Stiftung	Entwicklung eines in-vivo-Bioreaktors zur Reepithelial-isierung einer tissue-engineerten Neo-Trachea	PD Dr. Hillinger
Deutsche Forschungs-gemeinschaft	Entwicklung eines Modells der chronischen Abstossung nach Lungentransplantation	Dr. Jungraithmayr
SNF	Trachea reconstruction using novel tissue engineered constructs	Prof. W. Weder
Karitative Stiftung Dr. Gerber-ten Bosch, Zürich	- „Erweiterung des Organspendeangebotes von Lungentransplantationen“ LuTPL Pig	PD Dr. I.Inci
Sophien-Stiftung	Activity based protein profiling in human lung cancer biopsies	Dr. phil.S. Arni

Urological Research

Grants	Title of Project	Project Leader
Hartmann Müller Stiftung	„Interferenz zwischen Krebszellen und adulten Stamm-zellen im Anwendungsbereich des Tissue Engineering“	Dr. M Stölting/ Dr. D. Eberli
SNF	„Generation of a Recombinant Vaccinia Virus encoding immunogenic BKV Large T antigen/p53 binding domains epitopes to promote the expansion of effector T lymphocytes across a wide range of MHC class I and II antigens in prostate cancer patients“	Dr. M Provenzano

Cranio-Maxillofacial Surgery Research

Grants	Title of Project	Project Leader
The Swiss Competence Centre for Materials Research and Technology (CCMX), Education and Research Unit (ERU)	“Three Dimensionally Designed Cell Cultures Consisting of Microstructured Cell-sheets and Polymer Layers for Tissue Engineering	Prof Janos Vörös Prof. Franz E. Weber
SNF	Functional testing of diarthrodial joint soft tissues with in vivo acquired anatomical and kinematic information.	Prof Luigi Gallo Prof. Franz E. Weber
Arbeitsgemeinschaft Osteosynthese (AO-Davos, Switzerland)	Small chemicals to enhance bone repair	Prof Franz E. Weber
SNF	Synthetic biomimetic hydrogels for dual delivery of growth factors and their enhancers	Prof. Franz E. Weber

Surgical Intensive Care Medicine

Grants	Title of Project	Project Leader
SNF	Improvement of therapy in patients with severe traumatic brain injury differential impact of local and systemic changes and routinely applied drugs	PD Dr. Stover

7. Awards 2008

- PD Dr. med. Isabelle Schmitt-Opitz: Fellowship Award European Society for Medical Oncology
- PD Dr. med. Isabelle Schmitt-Opitz: Brompton prize from the European Association of Cardiovascular and Thoracic Surgery
- Dr. Stefan Heinrich: Preis der Schweizerischen Gesellschaft für Chirurgie, SGC Kongress Basel
- Dr. Olivier de Rougement: Forschungspreis der Association of Research in Surgery, SGC Kongress Basel
- Dr. M. Hübner: „Zollikofer Preis“ der Schweizerischen Arbeitsgemeinschaft für Laparo- und Thorakoskopische Chirurgie, SGC Kongress Basel
- Dipl. phil. II Th. Reding: Basic Research Poster Prize, European Pancreatic Club, Poland
- Dr. Dörthe Schmidt: Best Presentation 2008, 7th Day of Clinical Research, Zurich, Switzerland
- Dr. Alberto Silva: Poster prize, Day of Clinical Research, Zurich
- Dr. Mickael Lesurtel: Academie Francaise de la Médecine

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