Division of Surgical Research

Annual Report 2009

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 2009 of the Division of Surgical Research at the Department of Surgery, University Hospital Zurich.

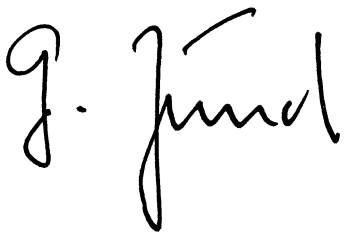
Among the personnel changes within the Department of Surgery, I have to mention the departure of Prof. Reto Stocker, Director of the Surgical Intensive Care Medicine at the end of December 2009. I would like to thank him for his personal support of the surgical intensive care research group.

The investments of laboratory equipment made in the past year include the purchase of a real-time PCR system, two water purification systems, a Western Blot system, two CO2 incubators, a streamer, two centrifuges, a laminar flow cabinet as well as a paraffin dispenser and an isoflurane vaporizer for the microsurgery and histology rooms and a digital camera and a thermal binding machine for our photo and graphic services.

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

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

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

Prof. Dr. med. Volkmar Falk Director Clinic of Cardiovascular Surgery

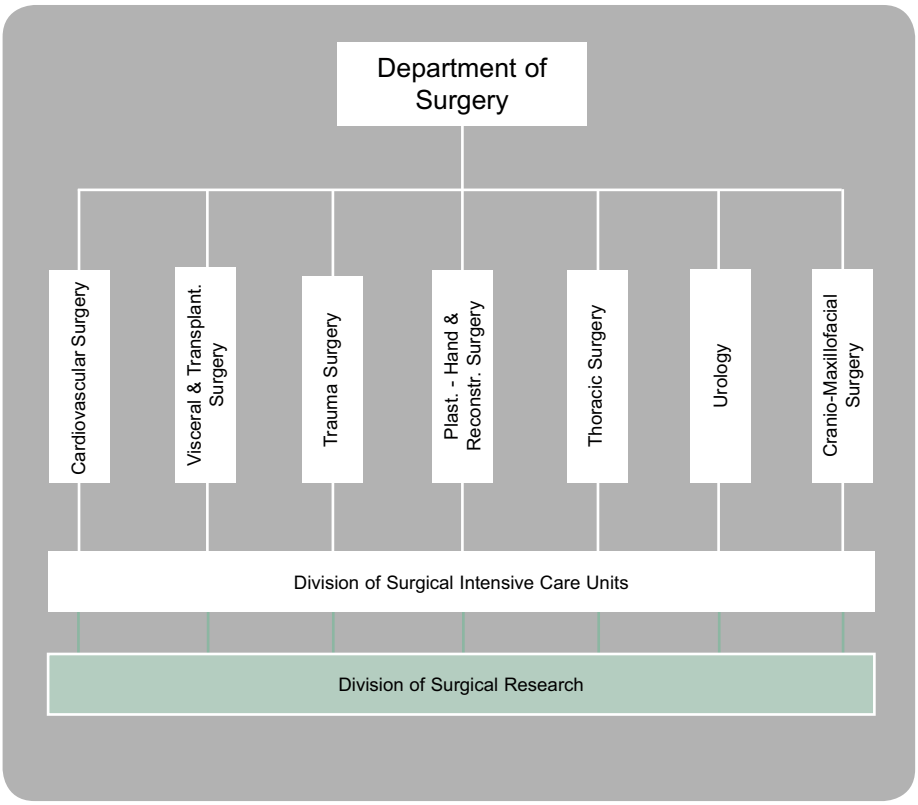
Prof. Dr. med. Pietro Giovanoli, Director Clinic of Plastic - Hand & Reconstr. Surgery

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

Prof. Dr 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

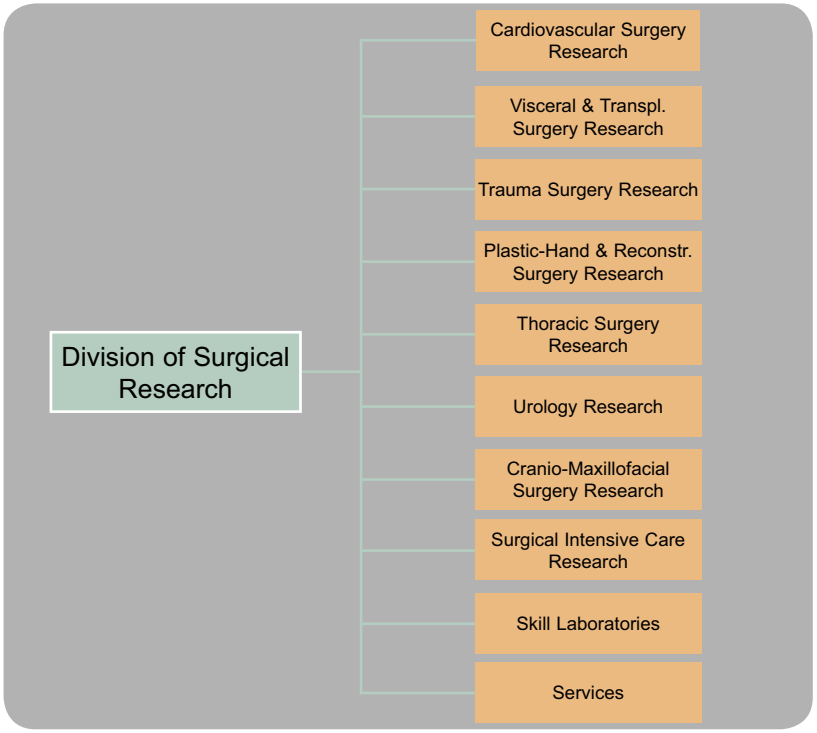


1.2 Structural Organisation of the Division of Surgical Research

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

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

Susanne Frehner,
Administration
Division of Surgical
Research

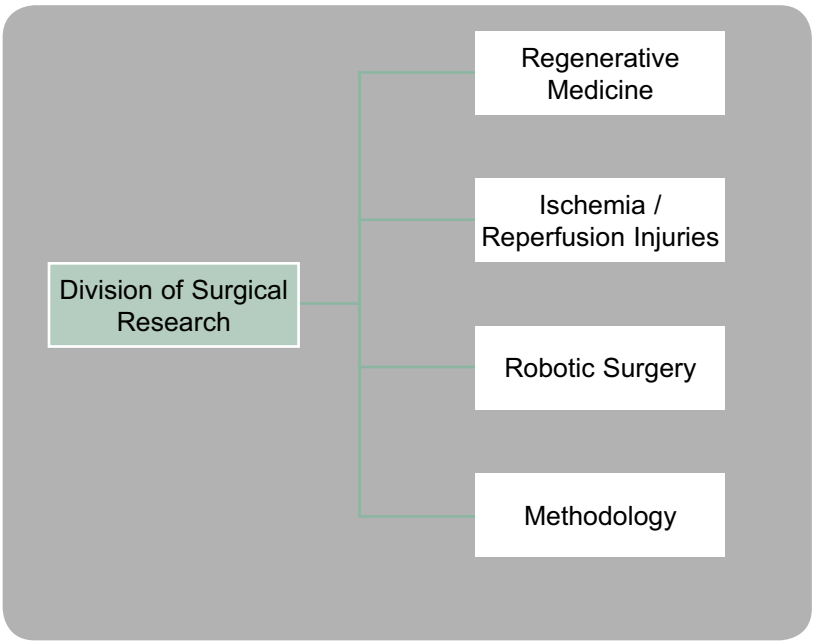


1.3 Scientific Sections within the Division of Surgical Research

Prof. Dr. med.
Simon Philipp
Hoerstrup
Regenerative
Medicine

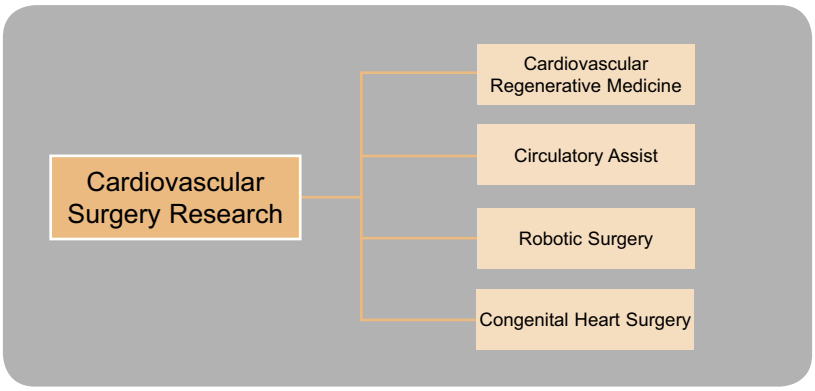
Dr. rer.nat.
Wolfgang Moritz
Ischemia /
Reperfusion
Injuries

Prof. Dr. phil II
Rolf Graf
Methodology



2. Research and Development

2.1 Cardiovascular Surgery Research




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


Prof. Dr. med. Volkmar Falk


Marion Fischer
Study Coordination and Administration


Sandra Geissler
Study Coordination and Administration

2.1.1 Cardiovascular Regenerative Medicine

Hoerstrup SP

1 Tissue Engineering and Cell Transplantation


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


Dr. sc. nat. Jens Kelm


Dr. sc. nat. Irina Agarkova


Dr. sc. nat. Roman Schönauer


René Stenger
Bachelor Chemistry


Eefje Bierhoff
med. techn. Assistentin


Dr. med. Max Emmert


Dr. med. Jan-Karl Burkhardt


Ursula Steckholzer
dipl. biomed. Analytikerin


Petra Wolint
Laborantin


Master Student Sarah Ronken


cand. med. Michael Bullen


cand. med. Chad Brokopp


med. Phd Student Benedikt Weber


Phd Student Jérôme Robert

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.

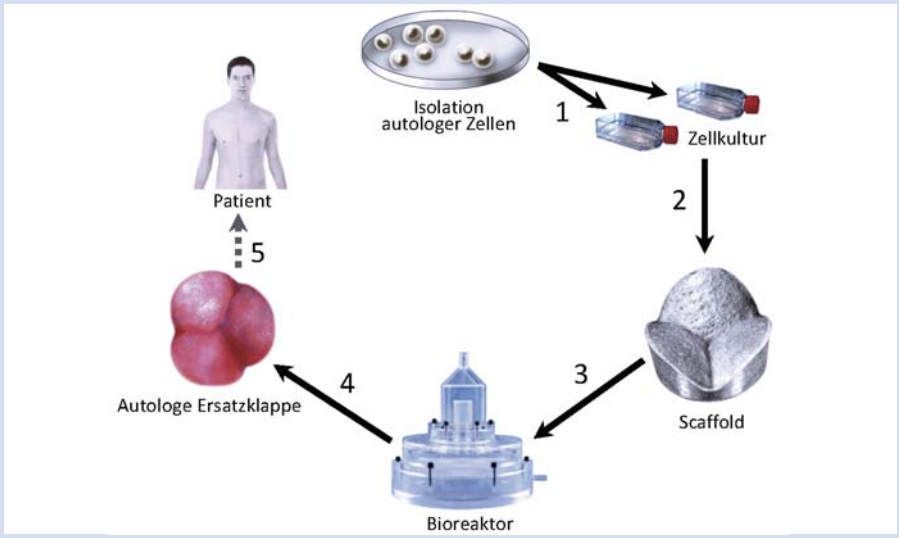
Research projects:

- 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.
Simon Schams

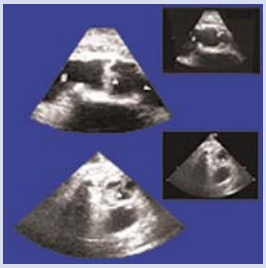
cand. med.
Pascal Heye

cand. med.
Karim Saba

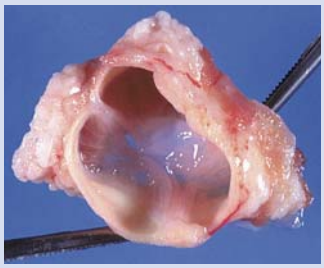


Proof of Heart Valve Tissue Engineering Concept

pre-implantation



in-vivo

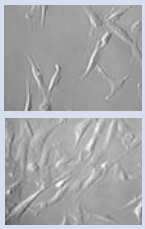


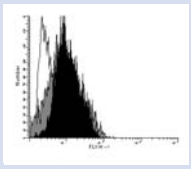
post-explantation

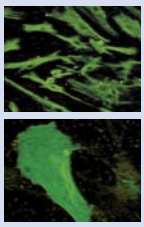
Autologous living tissue engineered heart valve in a sheep model, based on vascular - derived myofibroblasts and endothelial cells

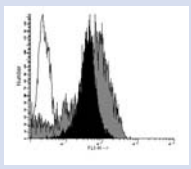
Hoerstrup et al. Circulation 2000

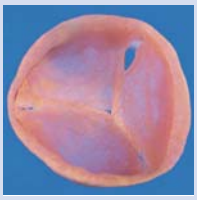
Human Heart Valve Tissue Engineering











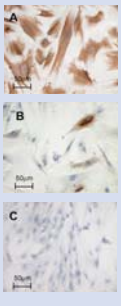
Heart valve tissue engineered from human marrow stromal cells

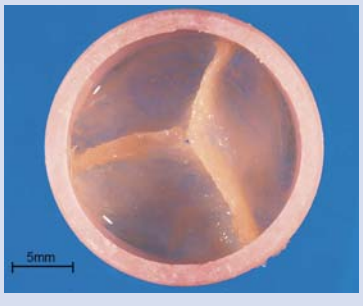
Hoerstrup et al. Circulation 2002

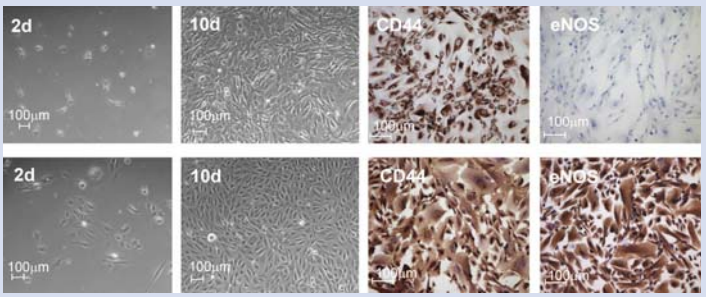
Human Prenatal Stem Cells for Pediatric Cardiovascular Tissue Engineering

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



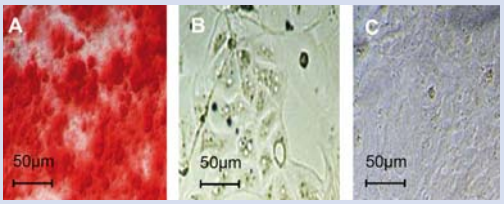




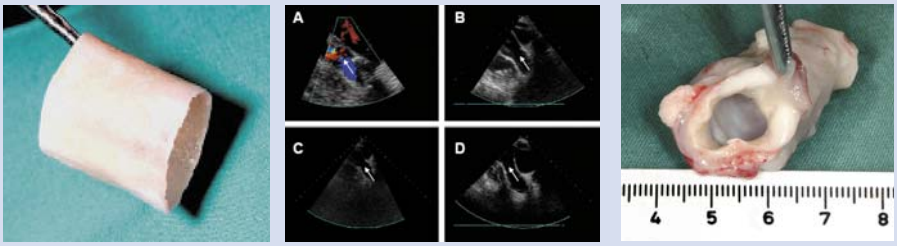
Fetal amniotic fluid-derived CD133+ and CD133- cells for autologous peditaric heart valve tissue engineering.
Schmidt D et al. Circulation 2007

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

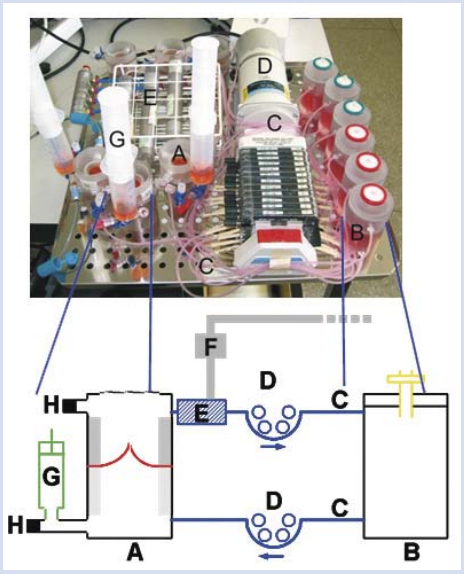


pre-implantation in-vivo post-explantation

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

Hoerstrup SP et al. Circulation 2006

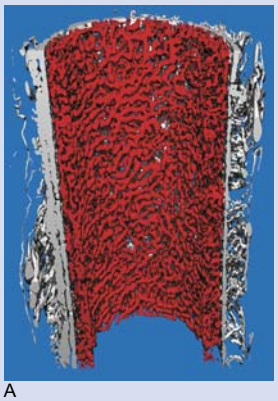
Bioreactor Development



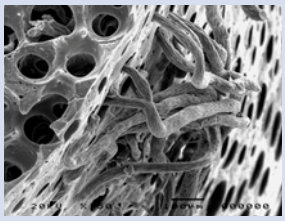
- sized small
- biocompatible materials
- maintenance of sterility
- static conditioning by continuous perfusion (4ml/min)
- dynamic conditioning by mimicking diastolic phase

Mol et al. Ann Biomed Eng 2005

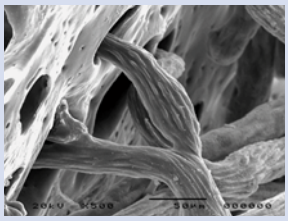
Neovascularisation of Biomaterials through Growth Factor Delivery



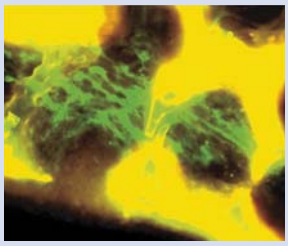
A



B



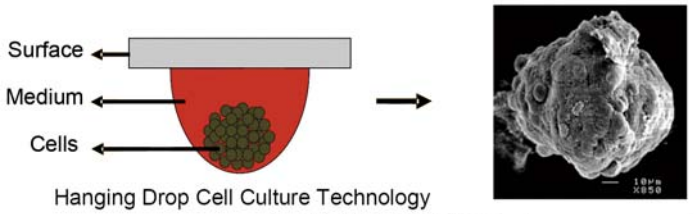
C

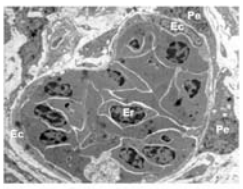


D

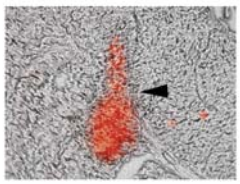
Increased Neovascularisation of porous Polyurethane as future bypass graft material induced by Vascular Endothelial Growth Factor (VEGF). Quantification of micro vascular network by Micro-CT (A), Corrosion Casting (B, C) and Lectin Perfusion (D)
(*Schmidt D et al.*) *unpublished data*

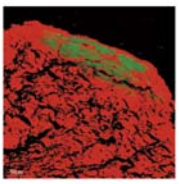
Design of Microtissues for Myocardial Regeneration





Functional cappillary formed in a myocardial microtissue





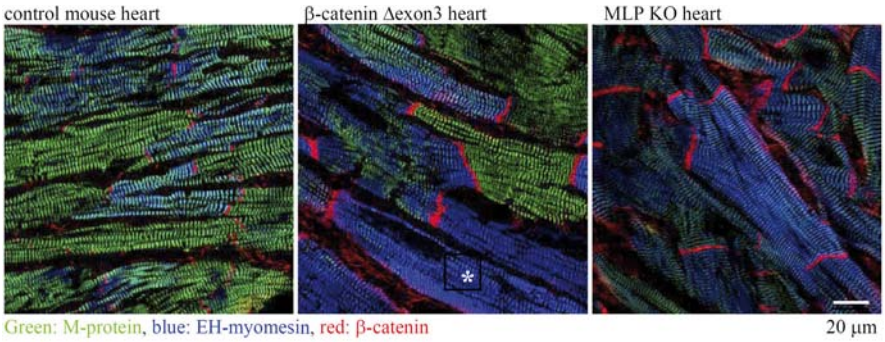
Fluorescent-traced microtissue integrated into the host myocardium.

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

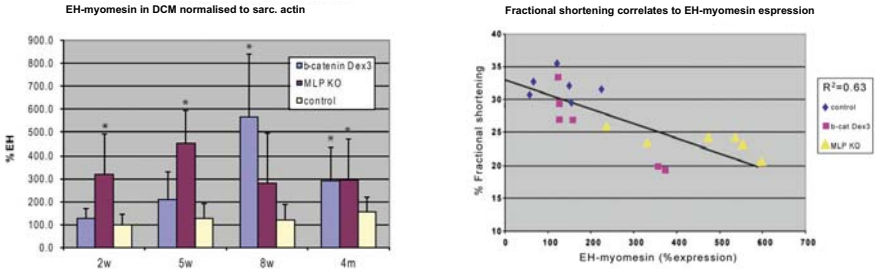
2 Cell based Regeneration

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).

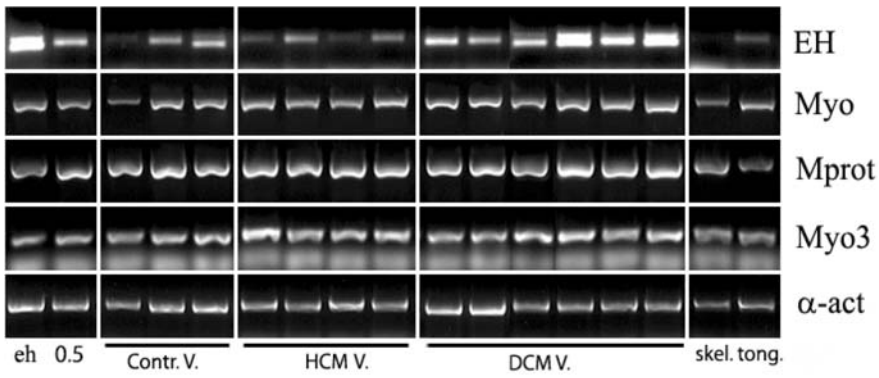


The diseased hearts are characterised by size irregularities of the cardiomyocytes and higher heterogeneity of the cytoskeletal components. Some cells change the M-band protein expression to a completely embryonic phenotype (, blue staining). In addition, intercalated disk proteins (e.g. β-catenin in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



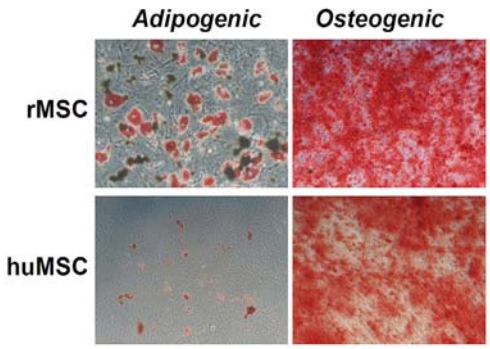
RT-PCR analysis of human patients suffering from DCM or HCM using primers specific for myomesin, M-protein and myomesin-3. Myomesin is evenly expressed in the heart samples (Myo) whereas the EH-myomesin isoform (EH) is upregulated in the dilated ventricles (DCM V.) up to levels comparable to embryonic heart (eh). No significant differences are detectable in the expression of M-protein (Mprot) and myomesin-3 (Myo3). α-actinin was used as loading control (α-act). 0.5=ventricle of child (age:0.5 years), Contr.=control, V.=ventricle., skel.=skeletal muscle, tong.=tongue.

Schoenauer et al., unpublished data

Isolation, expansion and characterisation of human MSCs

A major focus of our research is the investigation of human MSC's with regard to their regenerative capacity for myocardial repair and regeneration. Clinically relevant protocols for the isolation and processing of human bone-marrow derived MSCs are under investigation. Bone-marrow aspirates are obtained from routine surgical procedures involving exposure of the sternum, after informed consent. The aspirates are processed by Ficoll gradient centrifugation to isolate the mononuclear fraction using GMP compliant technologies. The resulting cell population is seeded into the culture flasks and the MSCs are selected due to their adhesive properties. The resulting cell population is expanded and analyzed by flow cytometry for MSC-positive and MSC-negative antigens (Figure 1, left panel). The multilineage potential of MSCs is confirmed by differentiation assays (Figure 1, right panel).

Surface Marker	huMSC
CD15 / SSEA-1	0%
CD31	0%
CD34	0.30%
CD44	62%
CD45	0%
CD90	99%
CD105	87%
CD106 / Vcam1	0%
CD146	38%
Flk-1	3.8%
GD2	2.4%
Stro-1	0%



Characterisation of human bone-marrow derived MSCs. Left panel: the representative human MSCs (passage 2) marker profile were analysed by flow cytometry. The MSCs quality is confirmed by higher expression of the CD105 and CD90 antigens and low expression of the CD45 and CD 34, specific for hematopoietic cells. Right panel: rat and human MSCs were induced along adipogenic (left, Oil Red staining) and osteogenic lineage (right, Alisarin Red staining). The results kindly provided by Petra Wolint

3 Disease Modeling

HDL transport through endothelial cells

Atherosclerosis is a chronic disease characterised by lipid retention and inflammation in the arterial intima. High density lipoproteins (HDL) and its major apolipoprotein (ApoA-I) exert diverse potentially atheroprotective functions: For example, they reduce oxidative damage, correct endothelial dysfunction, inhibit inflammation and mediate reverse cholesterol transport. The latter process involves the removal of excess cholesterol from peripheral tissues including macrophage foam cells in the arterial wall and its delivery to the liver for biliary excretion.
Many anti-atherogenic functions of HDL must be exerted in the arterial wall rather than in the plasma compartment. Therefore HDL or apoA-I must pass the endothelial barrier to get access to the foam cells, a process which is not well unterstood so far. In addition a functional blood vessel wall consists of interacting smooth muscle and endothelial cells.

The clinical chemistry group showed in cell culture experiments that endothelial cells transcytose apoA-I and HDL by distinct specific mechanisms involving the scavenger receptor SR-BI and the ATP binding cassette transporters ABCA1 and ABCG1.
The Hoerstrup's group demonstrated that it is possible to engineer functional artery equivalents with vascular cells. This raises the question, whether vascular grafts can serve as a model to study apoA-I/HDL transport in physiological and pathophysiological conditions.

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.

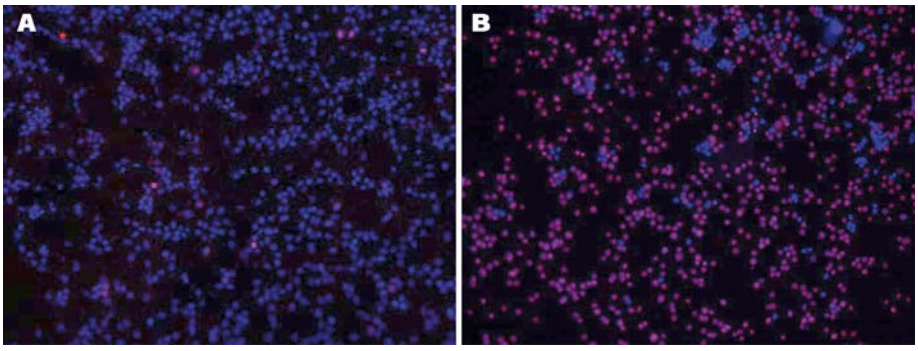


Figure 1: Cells seeded onto electrodes were stained with propidium iodide (red) to indicate membrane rupture and therefore death, and counterstained with DAPI to show living cells (blue). A) Control electrode without current after twelve hours. B) Working electrode after twelve hours)

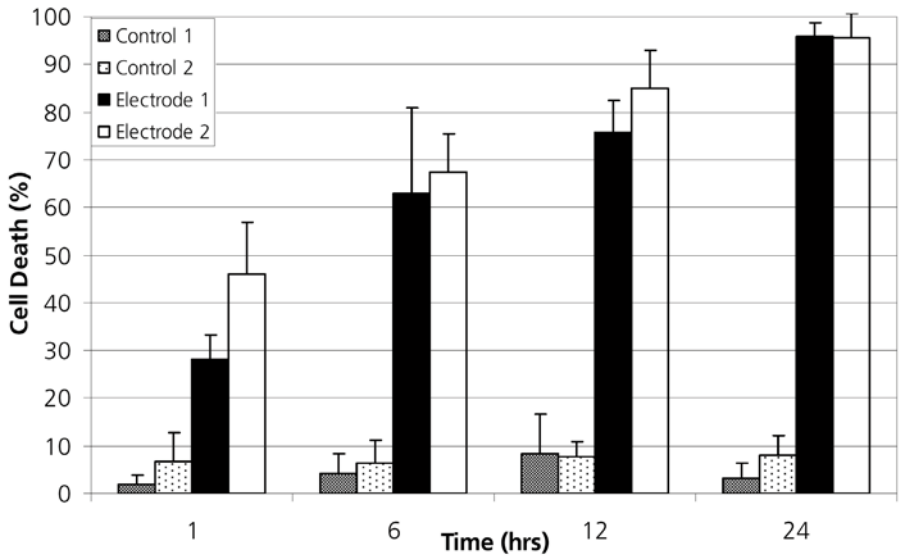


Figure 2: Summary of quantitative assessment of cell death

Achievements 2009

- Isolation und Charakterisierung von fetalen Progenitorzellen für die Forschung in der Regenerativen Medizin; Schweizer Nationalfonds, 2009 - 2012.
- 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.
- EU grant (FP7): „ Living autologous heart valves for minimally invasive implantation procedures". Seventh Framework Programme: Health-2009-1.4.2. Leading house: Regenerative Medicine Program, University Hospital Zurich (Prof. S. P. Hoerstrup). Coordination: Dr. R. Schönauer. 2009 - 2014.
- SNF grant SPUM (Special Program University Medicine): "Advanced Cell-Based Therapies for Cardiac Repair". Leading house: Regenerative Medicine Program, University Hospital Zurich (Prof. S. P. Hoerstrup). Coordination: Dr. Irina Agarkova.

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2.1.2 Mechanical Circulatory Support

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Long-term support

In patients with isolated left heart failure, we have used the Berlin Heart INCOR, a magnetically suspended and intracorporeally implanted axial-flow pump. It has been implanted in 15 patients so far. (fig. 1). Eight patients could be treated as outpatients, and three patients went back to work while being on support. Of the 15 patients, 10 (67%) were treated successfully. Nine of them were transplanted successfully, and in a young mother of two kids, the device could be explanted. One year after device removal, this patient is living a normal life.
The Berlin Heart EXCOR is an extracorporeally located pulsatile pump (fig. 2). It is used for biventricular or univentricular support. Until end of 2009, 22 patients were supported with the EXCOR. More than half of the patients (n=13) were discharged home with the device while they were waiting for heart transplantation, three of which went back to work and school, respectively. Fifteen patients (68%) were treated successfully, of which 14 were transplanted and one was weaned from the device.

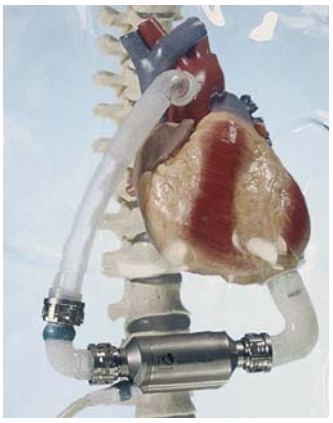


Figure 1 Berlin Heart INCOR (intrakorporale Lage)

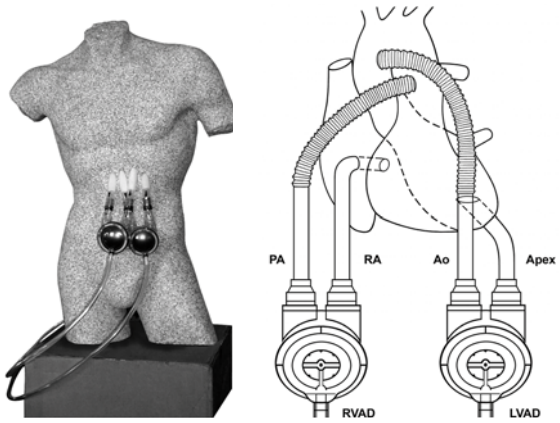
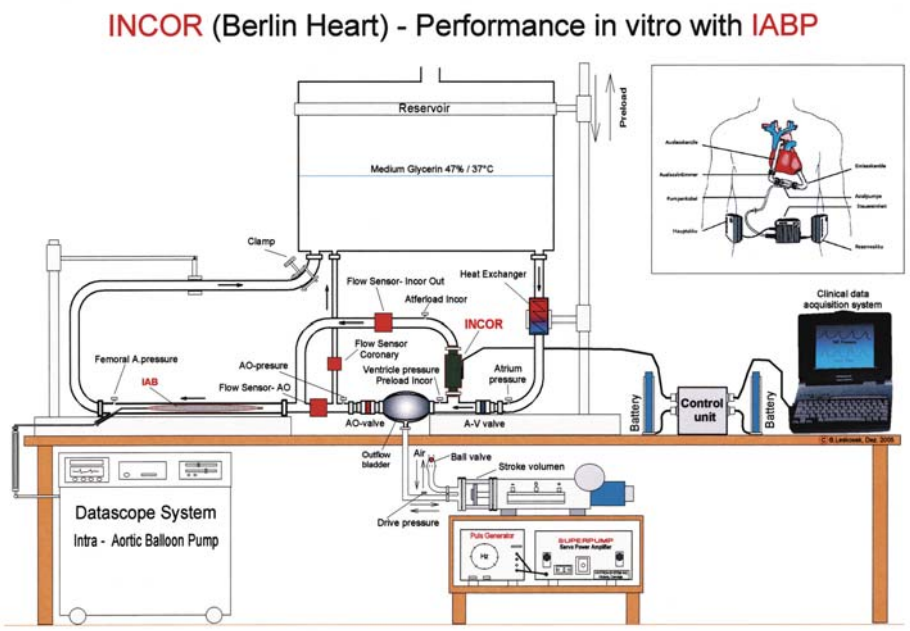


Figure 2 Berlin Heart EXCOR (links: extrakorporale Lage, rechts: Implantationsprinzip)

Short-term support

For short-term support, the ECMO (extracorporeal membrane oxygenation) was used. In 2009, more than 40 patients were supported with this device. ECMO support extended up to more than 4 weeks with good mechanical reliability.
In acute heart failure, veno-arterial ECMO 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 sepsis and fulminant pneumonia. Two patients received the ECMO for treatment of H1N1-pneumonia.
In 2009, we started a programm of ECMO support in peripheral hospitals. Four patients were implanted with an ECMO for acute lung failure at a local site and transported on ECMO to our center. Three of those patients could be salvaged.



Achievements 2009

- 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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2.1.3 Robotic Surgery and Innovative Technologies

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LAA Occlusion: Experience with a new clip device
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Left atrial appendage exclusion is a key aspect of the original Maze procedure for the surgical treatment of atrial fibrillation (AF). For this it was proposed to eliminate the LAA, as it was thought to be the source of many foci initiating AF in addition to being the origin of the majority (>90%) of strokes in AF patients[1]. This solution, while intuitive, was never independently studied and therefore Coumadin has remained the Gold standard for Patients with AF. It is well established that maintaining effective anticoagulation is very difficult and its use is associated with numerous and significant risks.

A recent study (Protect AF) of a percutaneous device to exclude the appendage proved the non-inferiority of appendage exclusion to Coumadin. The device was not without risk, as it reported a number of failed attempts to place the device and a concerning number of adverse events, in addition it was recommended that a cardiac Surgery suite be available when attempting this procedure[1].

Current surgical techniques to exclude the appendage (suture and stapling) have been shown to be frequently unsuccessful when subsequently evaluated by Echocardiography (TEE)[2]. In open cardiac surgery (Sternotomy) many surgeons do not address the LAA. This has two reasons, first the indication for LAA occlusion is not clear yet, second the techniques to obtain LAA occlusion remain limited and not without risk. These risks are mainly composed of bleeding and incomplete closure. With the increasing adoption of minimally invasive approaches to treat AF, options to effectively and safely exclude the LAA from the circulation remain limited.

The Gillinov-Cosgrove Clip© will be the first device specifically designed to surgically exclude the LAA. Currently, the devices used extended from other applications (staplers from general surgery, endo-loops from vessel occlusion) and are not specifically designed for this structure. This new device con-siders the frailty of the LAA, is designed to create immediate occlusion upon placement, comes in multiple sizes consistent with the breadths of tissue, allows repositioning to assure placement at the base of the structure, and eliminates the LAA without violating the tissue integrity via cutting or piercing. The clip generates a force profile specific to the tissue thickness and follows the natural orientation of the structure. Further it is designed to yield optimal endocardial remodeling allowing the body to fully reabsorb the LAA structure over time[3].

The device has yielded multiple research publications for the extensive preclinical research performed. Research was done at the Cleveland Clinic Foundation (led by Dr. M. Gillinov using canine model)[4], Emory University (led by Dr. J. Puskas using porcine model), the University of Cincinnati (led by Dr. J.M. Smith using porcine model). Additional chronic animal data and more insight into growth patterns was obtained in primates at Mount Sinai Medical Center, New York (led by Dr. S. Salzberg using papio model) [3].

With these data in hand, a first in human trial was initiated at the University of Zurich. Patients undergoing elective cardiac surgery were enrolled and received the Gillinov-Cosgrove Clip© as an adjunct to their ablation for AF. Currently 40 patients underwent Clip placement, and 2-year Follow-up is being completed. During clinical and computed tomography (CT) follow up after discharge, safe and effective LAA occlusion was documented in all patients [5]. In addition no neurological events (Stroke or TIA) occurred.

In light of the Protect AF data, demonstrating non inferiority for LAA device occlusion compared to warfarin, it appears that LAA occlusion will become a more established part of AF therapies. More randomized controlled data is necessary to establish LAA occlusion as a new indication in cardiac surgery. The Gillinov-Cosgrove Clip© is now available with CE Mark (Figures 1 & 2), and will provide an important tool for cardiac surgeons to achieve safe and durable LAA occlusion.

Figure 1

Figure 2

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- Laboratory for Thermodynamics in Emerging Technologies, ETH Zürich (Prof. Dimos Poulikakos)

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2.1.4 Congenital Heart Surgery

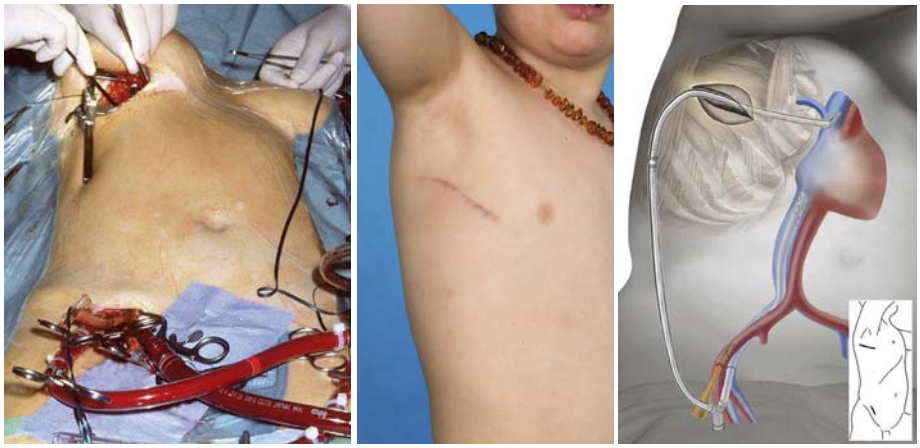
Prof Dr. med.
René Prêtre

Dr. med.
Hitendu Dave

Prêtre R, Dave H

Minimally invasive approach

The Division of Congenital Cardiovascular Surgery at the University Children's Hospital pursuing its efforts towards minimal invasiveness, both with regards to cosmetic mini-approaches, and the development of surgical instruments to achieve this goal. Many surgical procedures including ASD primum and secundum closure, mitral valve cleft closure, correction of partial anomalous right sided pulmonary venous connection, VSD closure, etc. have been adapted to the minimally invasive approach. As an extension of this approach, a first extrapleural ASD closure through a right axillary incision was recently performed, further extending the minimally invasive concept while achieving superb cosmetic result.



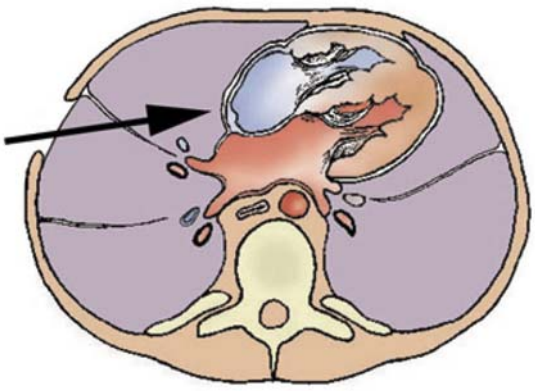


Figure 1

Collaterals after extrapleural approach

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 transpleural approach versus an extra-pleural approach. In spite of small numbers of patients which could be included based on our study criteria, there was a trend towards lesser collateral development with extrapleural approach.
Attempts are on to objectively estimate collateral blood flow as derived by MR estimates of pulmonary arterial from pulmonary venous flow.

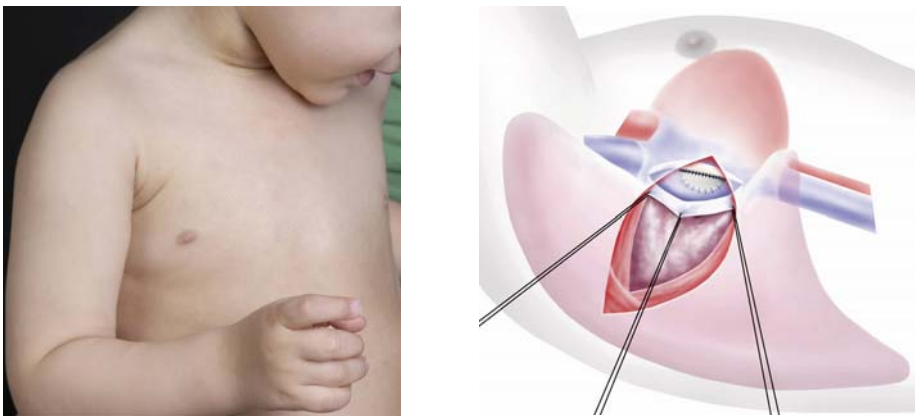


Figure 2

Ductal flow to perform aortic arch enlargement without cardiopulmonary bypass

Since nearly 40% of patients with neonatal aortic coarctation loco classico have variable degrees of aortic arch hypoplasia, a novel approach of using the ductal perfusion to the lower body while performing aortic arch enlargement (Fig 3), thereby avoiding the use of cardiopulmonary bypass with its attendant risks was developed and used effectively in 8 patients.

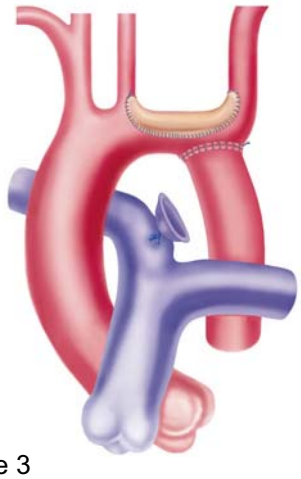


Figure 3

Left heart epicardial pacing

Advantages of left heart pacing on long term ventricular synchronization were documented. The implantation procedure was minimally invasive through a muscle sparing left axillary incision (Fig 4). Keeping in mind the increasing role of epicardial placement of definitive electrodes on the left ventricle, development of new electrodes for long-term intramyocardial ventricular stimulation is being pursued. A newly designed electrode, that could be inserted directly into the myocardium is expected to alleviate the problems of positioning the electrode on the heart surface masked by postoperative adhesions.

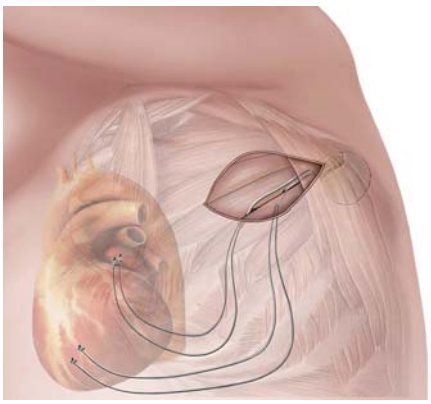


Figure 4

Cantrell's Syndrome

A neonate with Cantrell's syndrome with subcutaneously lying contractile extension of the left ventricular apex ending in a bullous extension under the umbilicus, was successfully excised and the anterior rectus wall was reconstructed with a overlapping Mayo plasty.

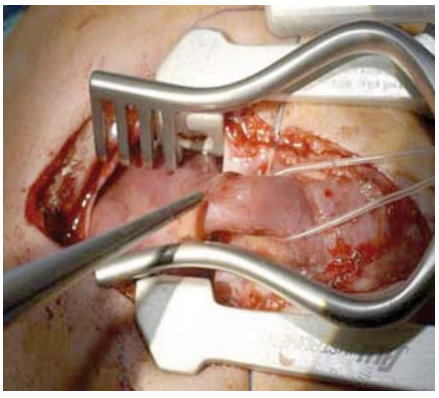


Figure 5

Pulmonary artery banding clip (patented)

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. A batch of 5 pigs have been subjected to banding using the above mentioned clip on the right femoral artery as well as a classic banding on the left femoral artery. Both the forms of banding with be removed after 6 weeks and the debanded arteries allowed to recover and grow for another 4 weeks before being subjected to histopathological examination (Fig 6).

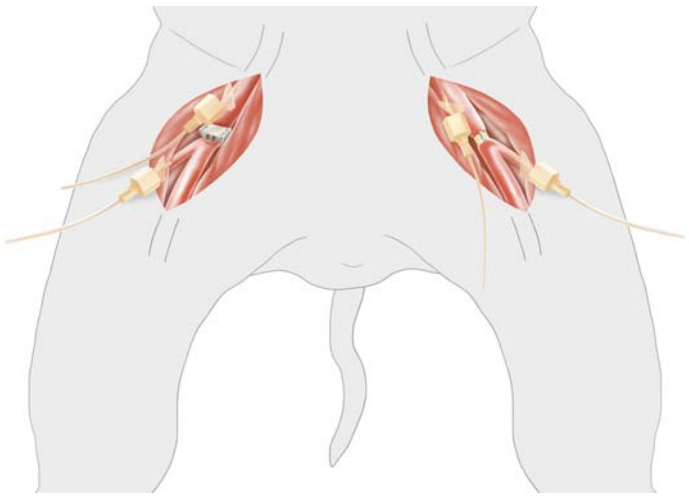


Figure 6

Achievements 2009

- 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

Prof. Dr. phil II
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Prof. Dr. med.
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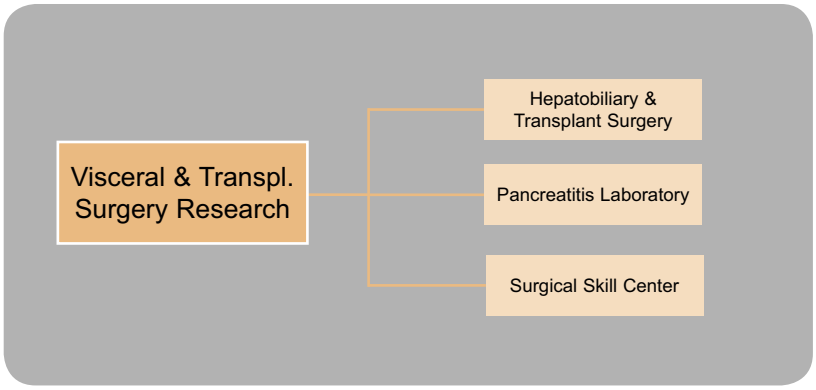
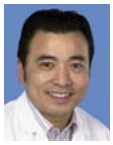


2.2.1 Hepatobiliary & Transplant Surgery



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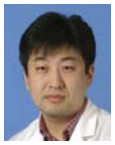
Yinghua Tian

Dr. sc. nat.
Wolfgang Moritz

Dr. med.
Andreas Rickenbacher

Dr. med.
Ashraf Osman



Dr. sc. nat.
Jae-Hwi Jang

Dr. med.
Christian Oberkofler

Dr. med.
Kuno Lehmann

Udo Ungethüm

Cholestasis inhibits liver regeneration
Rickenbacher A

Fasting and calorie restriction is associated with a longer life span and higher resistance to stress. This phenomenon is well studied in lower animals such as yeast, worms or flies but also seems to be applicable for mammals. In this project we study the influence of fasting on the injury after ischemia and reperfusion of the liver. Several studies have shown a protective effect in different organs. However, the underlining mechanism is poorly understood. To study this question a model of one hour 70% liver ischemia followed by reperfusion in C57BL/6 mice was used. While the control group had unlimited access to food, the other groups were fasted for 24, 48 and 72 hours prior to ischemia.

As expected there was a significant weight loss with duration of fasting. Interestingly, 24 hours of fasting resulted in less ischemia/reperfusion injury assessed by serum AST and ALT levels, injured area in H&E histology and TUNEL staining. (see Figure 1)

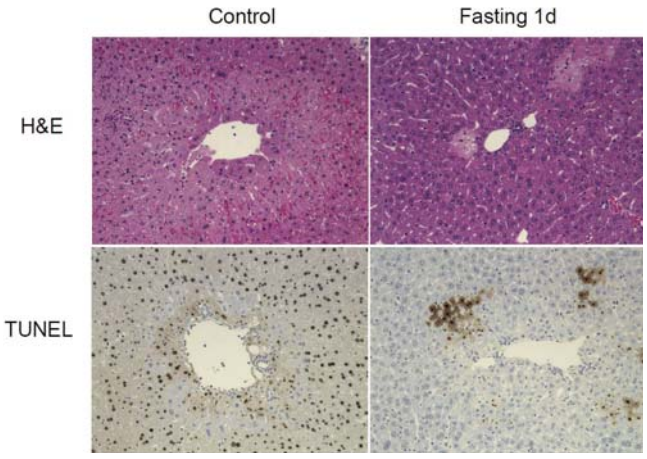


Figure 1: HE and TUNEL staining of control and one day fasting group after 1 hour ischemia and 6 hours of reperfusion.

Surprisingly, this protective effect was lost in groups that fasted for 48 and 72 hours. Fasting was associated with a loss of energy stores i.e. a decrease of serum glucose, serum triglycerides, liver glycogen, but and increase in lipid droplets within hepatocytes. (see Figure 2) Inflammatory cytokine expression levels were reduced in the 24 hours fasting group suggesting that the secondary inflammatory response is decreased. This could be substantiated with in vitro experiments. A macrophage cell line produced less TNFα after serum or glucose deprivation simulating fasting. (Figure 3)

These results suggest that fasting is protective in ischemia reperfusion. However, this is a transient effect and seems to involve inflammatory cells. Our goal is to further identify the key molecules that are involved in this protective effect.

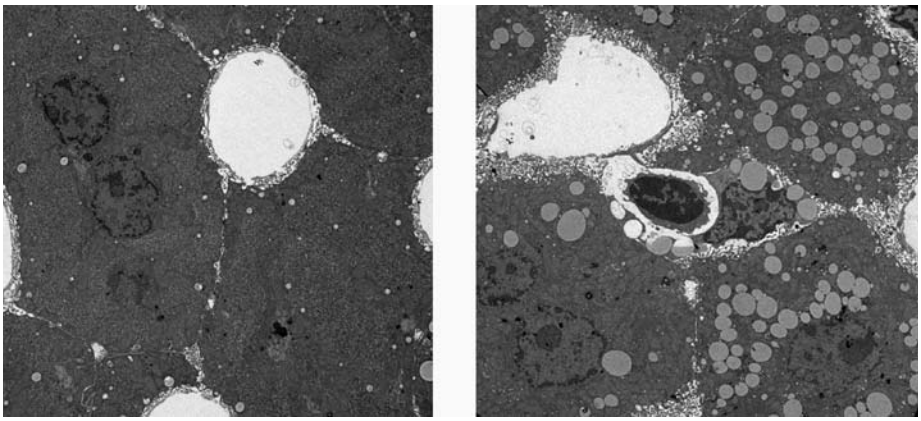


Figure 2: Transmission electron microscopy (TEM) of control (left) and one day fasting group (right) showing loss of glycogen and increase in lipid droplets.

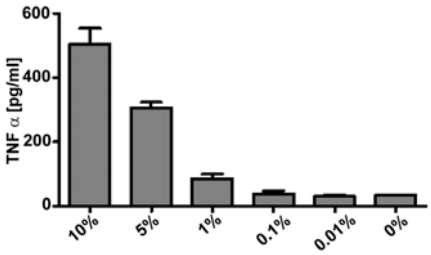
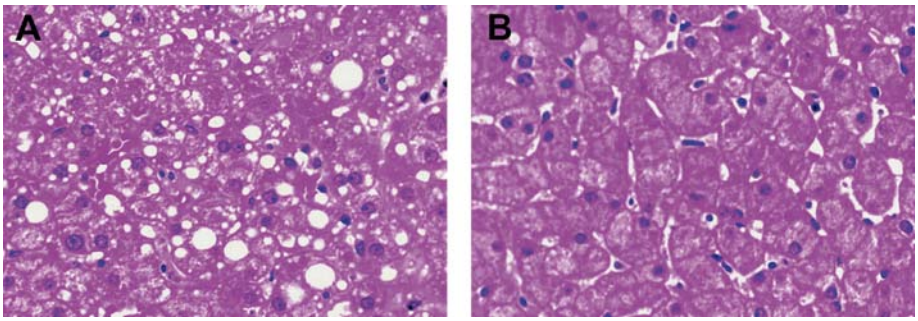


Figure 3: TNFα levels in supernatant of macrophage cell line incubated with different concentrations of FCS

Amelioration of hepatic steatosis in live liver donors by ω-3 fatty acids

Osman A, Breitenstein S

We have previously demonstrated that oral administration of ω-3 fatty acids decreases hepatic lipid content and ameliorates microcirculation in mice (1). Since the fatty liver is one of the most common “marginal grafts”; reduction of liver steatosis may help expand the donor pool. Recently, we have treated three moderately steatotic live donors with ω-3 fatty acids capsules with remarkable success.



Oral administration of ω-3 fatty acids for one month noticeably reduced the grade of steatosis from (A) 20-40% to (B) 10-20%. Liver sections were evaluated by the same pathologist.

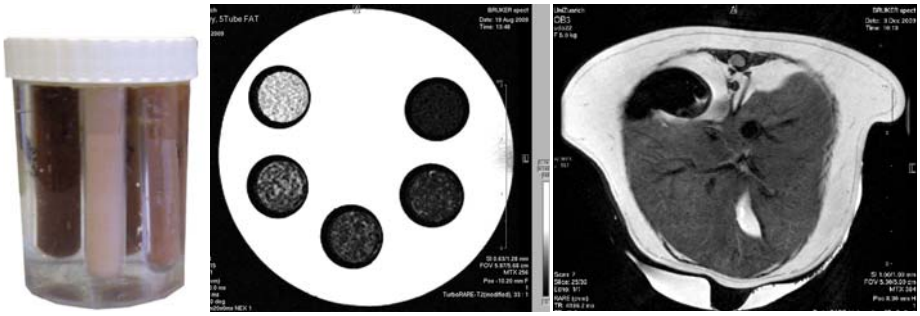
1. El-Badry AM, Moritz W, Contaldo C, Tian Y, Graf R, Clavien PA. Prevention of reperfusion injury and microcirculatory failure in macrosteatotic mouse liver by omega-3 fatty acids. Hepatology 2007.

Development and Validation of a "Phantom" for accurate assessment of hepatic steatosis with CT and MRI

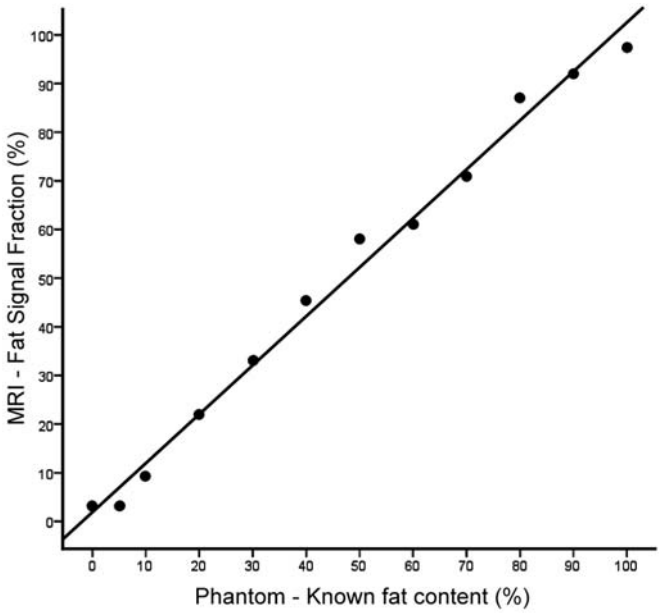
Raptis D, Osman A, Fischer M*

*Collaboration with Dept. Radiology

Non-alcoholic fatty liver disease (NADL) refers to a wide spectrum of liver injury, ranging from simple steatosis to cirrhosis. In addition, liver fat is a risk factor for postoperative complications in liver resection and liver transplantation. Within the search of a new Gold Standard for quantification of hepatic steatosis, non-invasive imaging modalities need to be evaluated for their clinical impact. We developed a robust preparation method of a phantom containing homogenized mixtures of liver tissue and fat at different concentrations in order to validate non-invasive methods of liver-fat quantification based on MRI or CT imaging.



The phantom with different amounts of homogenized liver/fat, its appearance on the Small Animal MRI, and validation by leptin deficient steatotic mice.



Correlation of known fat contents with those predicted by MRI.

Small for size syndrome in a murine model of extended liver resection
Lehmann K, Jang JH, Rickenbacher A, Tschopp O, Moritz W

After extended liver resections, patients may develop prolonged hyperbilirubinemia, deteriorated clotting times, and encephalopathy within the first days. These are symptoms of small for size (SFSS) syndrome, which carries a high risk for additional morbidity and mortality. Here, we aimed to setup a murine model for small for size syndrome after liver resection, excluding ischemia and reperfusion injury. We performed a standard partial (pHx, 70%) or extended (eHx, 90%, Fig1) hepatectomy in 10 weeks old C57BL/6 mice. During eHx, portal vessels of all hepatic lobes (excl. right posterior lobe) were selectively ligated. Extended hepatectomy provoked symptoms of SFSS, with a 10% mortality at 48h. These animals had higher serum bilirubin levels (10uM ± 1.5 vs. 2.6 ± 0.5, P=0.005). On histology, hepatocyte swelling and microvesicular steatosis was prominent (Figure 2). Although total ATP content was identical, hepatic lipid content increased. Furthermore, in animals after eHx the mitotic index was decreased (1.4 ± 0.5 vs. 5.0 ± 1.6, P=0.017, Figure 3). Extended hepatectomy in the mouse induces a small for size syndrome with prominent microvesicular steatosis, and reduced hepatocyte proliferation. Further exploration of this model may help to elucidate mechanisms leading to small for size syndrome.

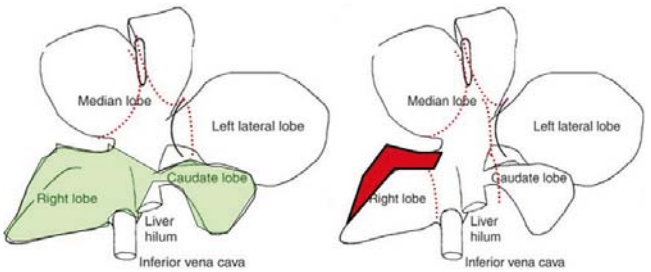


Figure 1: Microsurgical Model for SFSS

A: Standard 70% Hepatectomy: Ligation of the left and middle lobe. B: 90% Hepatectomy with additional ligation of the caudate, and anterior right lobes (remnants are coloured).

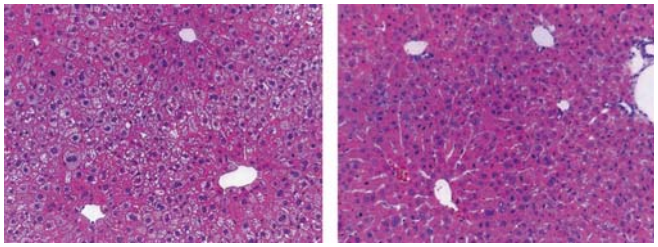


Figure 2: Liver sections (H&E) after 90% and 70% hepatectomy.

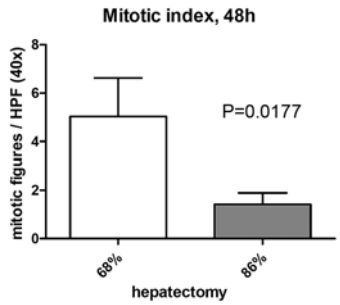


Figure 3: Mitotic index is decreased in animals after extended hepatectomy.

Targeting oxygen free radical levels: A novel strategy for radical cytoreduction during hyperthermic intraperitoneal chemotherapy (HIPEC)

Lehmann K, Rickenbacher A, Jang JH, Oberkofler C, Vonlanthen R, Graf R, Gertsch P

To tailor a cytotoxic therapy to cancer cells found in peritoneal carcinomatosis. Hyperthermic intraperitoneal chemotherapy (HIPEC) is a therapy complementary to extensive peritoneal excision of malignant tumors. HIPEC is performed after surgical cytoreduction by washing the peritoneum with a hyperthermic (42°C) solution containing mitomycinC/doxorubicin or oxaliplatin targeting residual tumor cells. However, many primary tumors respond poorly to these agents, therefore alternative approaches to target chemo-resistant cells are needed. Reactive oxygen species (ROS) are a novel approach to induce death of residual cancer cells.

A series of experiments demonstrated that hyperthermia alone at 42°C had little cytotoxic effect, but rapidly induced protective mechanisms, e.g. heat shock protein 70 (Fig 2). Oxaliplatin and mitomycinC/doxorubicin conferred inconsistent cytotoxicity depending on cell lines and doses. For example, 50% of HT29 cells were viable 7 days after hyperthermia with oxaliplatin. Low doses of hydrogen peroxide consistently activated apoptotic pathways with increased cell death in combination with HT (Fig2), but showed little toxicity at physiologic temperatures, and cytotoxicity was reversible after pretreatment with N-acetylcystein (Fig 1). The effect on HT29 and SW403 cells was significantly superior compared with mitomycinC/doxorubicin or oxaliplatin. This suggests that the combination of hyperthermia, together with low dose hydrogen peroxide, is sufficient to exert tumor cell death.

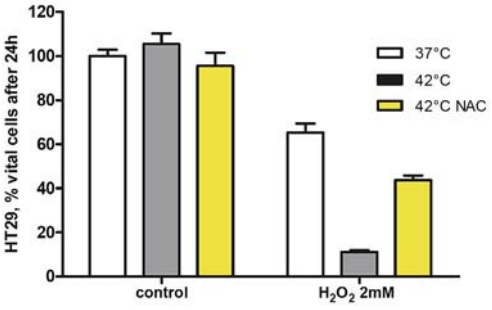


Figure 1: Survival of tumor cells after 90 minutes treatment at 42°C, and recovery in normal media for 24 hours. .

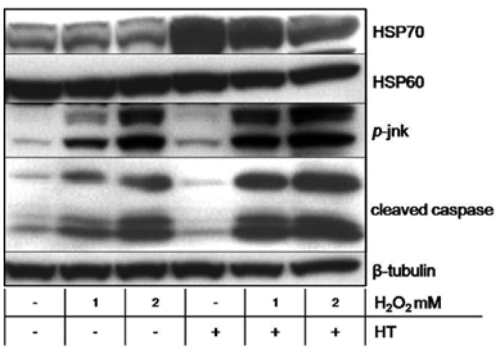
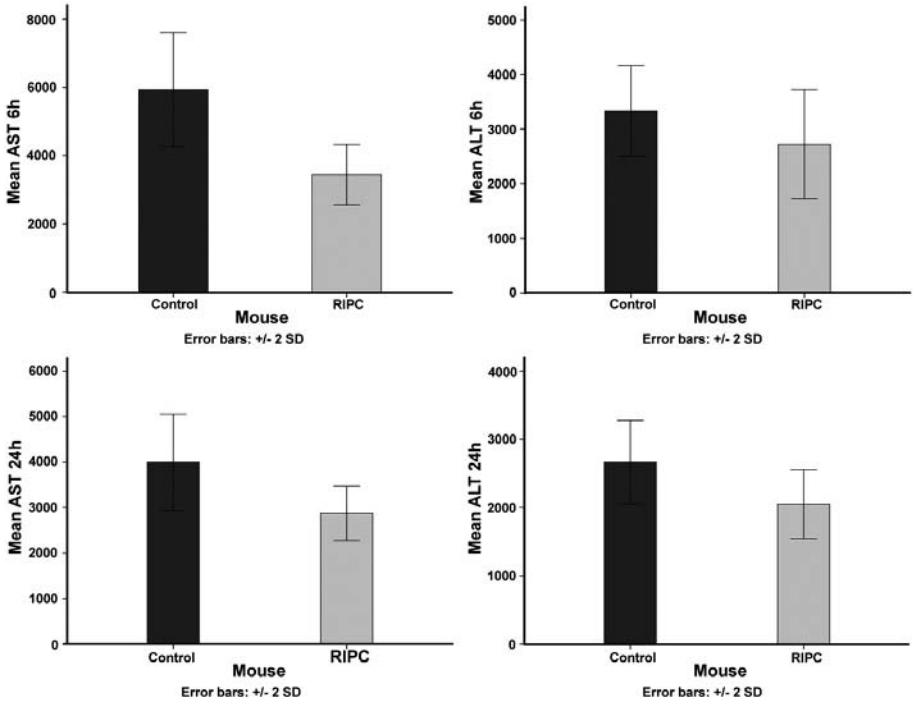


Figure 2: Hyperthermia induces Hsp70 without activation of Caspase3, whereas low doses of peroxide rapidly activated apoptotic signaling.

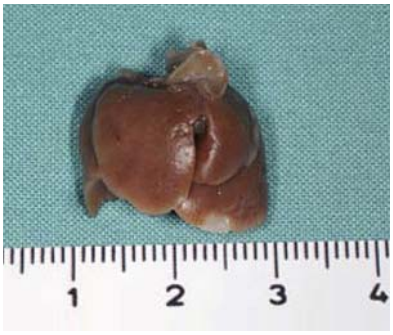
Remote ischemic preconditioning (RIPC) in the steatotic liver
Oberkofler C, Graf R

Ischemia reperfusion injury (IRI) is still a major concern in liver surgery and transplantation. The incidence of primary non-function in liver transplantation is 5-10%. In major liver resection the pringle maneuver to prevent blood loss leads to a sustained ischemia of the liver and subsequently reperfusion injury. This problem is even more severe in steatotic livers. Direct ischemic preconditioning (IPC) has shown to be cytoprotective and to reduce the severity of IRI. Ischemic preconditioning (IPC) is done by an interruption of the blood flow to the liver for brief periods followed by reperfusion. Unfortunately, a shortcoming of IPC is the trauma to the vessels and stress directed to the target organ. Remote ischemic preconditioning (RIPC) is a new technique where the ischemic preconditioning is performed in one organ and protection results in remote organs. It has been speculated that remote preconditioning acts by release of humoral factors into the circulation which then are protecting the remote organ. Experimental studies showed a cytoprotective effect to the heart, lung and kidney after brief periods of ischemia in the mesentery artery, the limb and kidney.
In our study we are focusing on the effect of RIPC in the liver, especially steatotic liver using the ob/ob mouse model. RIPC is done by 4 cycles of 5 minutes ischemia to the lower limb before a sustained 70% ischemia to the liver. Preliminary results could show a protective effect in the RIPC group. Further investigations will have a closer look at the underlying mechanisms.



5HT inhibits tumor growth in a liver specific tumor model.
Oberkofler C, Soll C

A subcutaneous tumor model is a surrogate model for hepatocellular carcinoma. Because the environment of the liver, with its complex composition of different cell types, can influence tumor growth and also change the effect of 5HT and those antagonists. We currently establish an intrahepatic tumor model. Preliminary experiments were done in a xenograft murine cancer model. We used stable tranfected luciferase HCT cells derived from human adenocarcinoma of the colon and injected the cells through the portal vein into the liver of 6 to 8 weeks old male nu/nu mice.
To monitor tumor growth, quantify tumor burden in the mouse liver and follow responses to eventually therapeutic treatments we used the non-invasive in-vivo bioluminescent imaging system (IVIS, Xenogen). After inoculation of $1x10^6$ HCT cells measurements were performed to assess tumor growth (Figure 1). Bioluminescent imaging of tumor growth allows a longitudinal evaluation of tumor development before, during and after treatment, offering an excellent preclinialstrategy to assess tumor response. Now we want transfect human hepatocellular cancer cell lines with the luciferase gene in order to use the bioluminescent imaging. Within this new model, we are planning to test an orally active antagonist against the 5HT2B receptor, LY 272015 hydrochloride. An animal group treated with rapamycin and appropriate vehicles shall serve as control. We also want to harvest different organs and blood to perform toxicological studies. These experiments are already proven by the cantonal veterinary office of Zurich.

A 

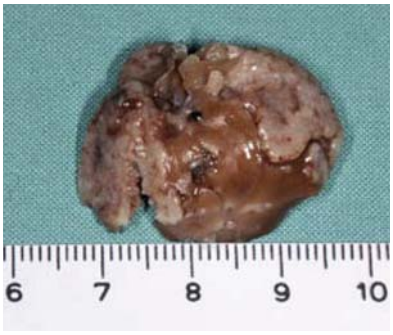
B 

Figure 1: HCT cells were stable transfected with a luciferase reporter gene and $1x10_6$ cells were injected through the portal vein of nude mice. Tumor growth was monitored with an in-vivo bioluminiscent imaging system after intra-peritoneal injection of 1 fg/g BW luciferin.

A: Normal mouse liver **B**: Mouse liver 10 days after injection

PD Dr. med.
Phillipp Dutkowski

Dr. med.
Olivier de Rougemont

Transplantation and perfusion
Hypothermic oxygenated perfusion of liver grafts
Dutkowski P, de Rougement O

Our group previously showed in various rodent models the benefit of endischemic hypothermic oxygenated perfusion (HOPE) in protecting livers donated after cardiac death (DCD livers)(1-2). In addition, we demonstrated recently in a large animal transplant model, that short term HOPE appears as an effective and practical strategy for severely injured pig liver grafts. One hour HOPE treatment after 60 minutes in situ ischemia followed by 6 hour cold storage led to improved survival after transplantation. In contrast, untreated liver grafts caused repetitive primary non function after implantation (3). In a next step, we tested if machine perfusion without oxygen would prevent the protective effect of HOPE. One hour perfusion with a completely deoxygenated perfusate (HnOPE) caused a high amount of released AST due to necrosis, similar to untreated livers (Figure 1A&B). Having shown, that perfusate oxygen was one major component responsible for protection of DCD grafts during hypothermic perfusion, we tested, if hepatocyte mitochondria appear as the primary target. For this purpose, we added cyclosporine (5µM CsA) to the deoxygenated perfusate. This drug is known to inhibit the opening of the mitochondrial permeable transition (MPT) pore, thus preventing the release of mitochondrial cytochrome c. One hour perfusion with deoxygenated perfusate in the presence of cyclosporine (CsA) showed a strong protective effect in terms of improved histology (Figure (1C) and low AST release (Figure 2).

Based on these results we believe that HOPE transmits its protective effects by preserving mitochondrial electron transfer during hypothermia. The oxygenation of mitochondria in the cold leads to a change in mitochondrial redox state before rewarming, resulting in prevention of MPT pore during reperfusion.

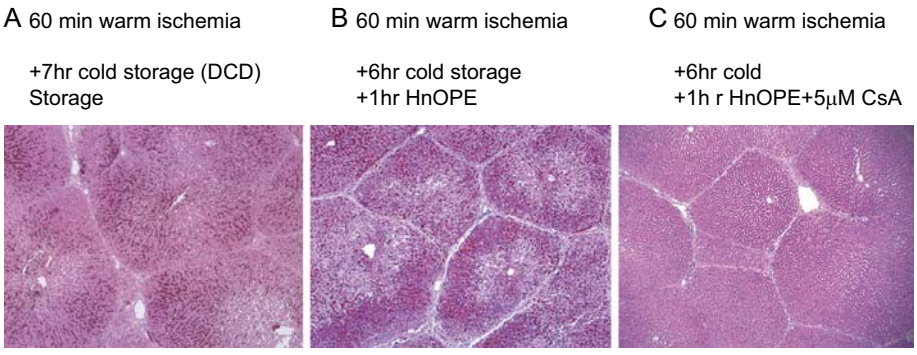


Figure 1: HE staining after 3 hours of ex situ reperfusion (isolated perfused liver)

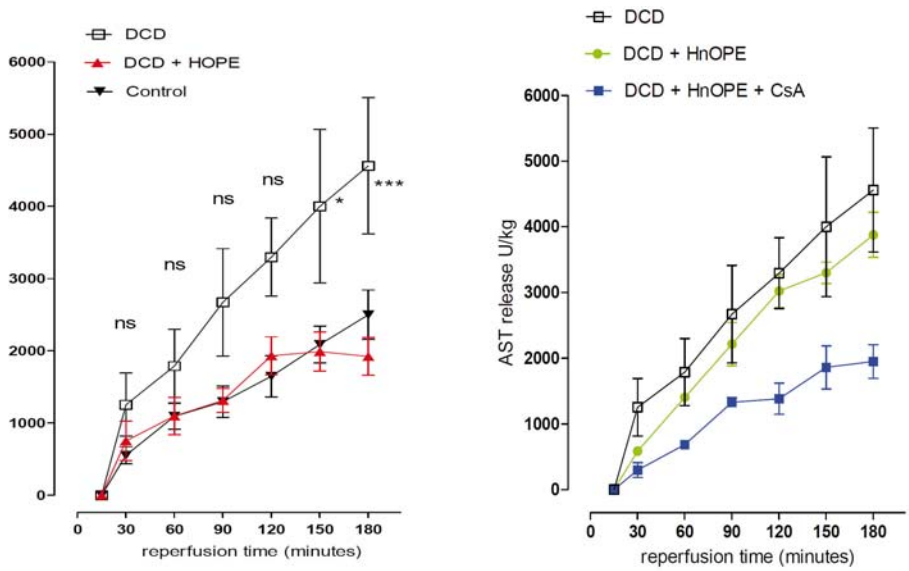


Figure 2: Hepatocyte injury (AST release) during 3 hours of ex vivo reperfusion (isolated perfused liver)

Achievements 2009

Findings:

- Fasting protects the liver from ischemia reperfusion injury
- Ex vivo hypothermic oxygenated perfusion (HOPE) improves graft function in a large animal liver transplant model.
- Remote ischemic preconditioning of an extremity protects the liver

Collaborations:

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

Selected references:

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- Dutkowski P, Furrer K, Tian Y, Graf R, Clavien PA. Novel short term hypothermic oxygenated perfusion (HOPE) system prevents injury in rat liver graft from non-heart beating donor. Ann Surg 2006, 244: 968-976.
- de Rougemont O, Breitenstein S, Leskosek B, Weber A, Graf R, Clavien PA, Dutkowski P. One hour hypothermic oxygenated perfusion (HOPE) protects nonviable liver allografts donated after cardiac death. Ann Surg. 2009 Nov;250(5):674-83.

Dr. med.
Christopher Soll

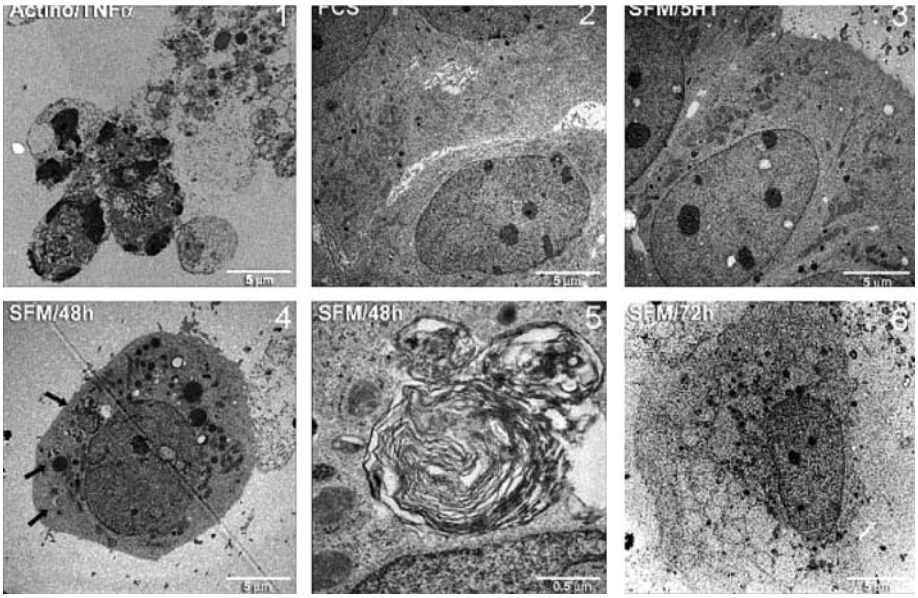
Dr. sc. nat.
Jae-Hwi Jang

Oncology

Serotonin suppresses autophagy in human hepatocellular cancer cells

Soll C, Dr. Jang JH, Oberkofler C

In addition to its function as a neurotransmitter and vascular active molecule, serotonin is also a mitogen for hepatocytes and promotes liver regeneration. A possible role in hepatocellular cancer has not yet been investigated. Human hepatocellular cancer cell lines Huh7 and HepG2 were used to assess the function of serotonin in these cell lines. Characteristics of autophagy were detected with transmission electron microscopy, immunoblots of microtubule-associated protein light chain 3(LC3) and p62 (sequestosome 1). Immunoblots of the mammalian target of rapamycin (mTOR) and its downstream targets p70S6K and 4E-BP1 were used to investigate signaling pathways of serotonin. Two different animal models served as principle of proof of in vitro findings. Clinical relevance of the experimental findings was evaluated with a tissue microarray from 168 patients with hepatocellular carcinoma. Serotonin promotes tumor growth and survival in starved hepatocellular carcinoma cells. During starvation hepatocellular carcinoma cells exhibited characteristics of autophagy, which disappeared in serotonin-treated cells. Rapamycin, an inhibitor of mTOR, is known to induce autophagy. Serotonin could override rapamycin by an mTOR-independent pathway and activate common downstream signals such as p70S6K and 4E-BP1. In two tumor models of the mouse, inhibition of serotonin signaling consistently impaired tumor growth. Human biopsies revealed expression of the serotonin receptor HTR2B, correlating with downstream signals, e.g., phosphorylated p70S6K and proliferation. This study provides evidence that serotonin is involved in tumor growth of hepatocellular cancer by activating downstream targets of mTOR, and therefore serotonin-related pathways might represent a new treatment strategy.



(A) Upon serum deprivation, human hepatocellular carcinoma cells (Huh7, HepG2) go through autophagy before cell death occurs. Electron micrographs of HCC cells treated with Actinomycin/TNFa (positive control), with full serum (negative control) , or in the absence of serum with or without serotonin.

Collaborations:

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

Selected references:

- Christopher Soll, Jae Hwi Jang, Marc-Oliver Riener, Wolfgang Moritz, Peter Johannes Wild, Rolf Graf, and Pierre-Alain Clavien Serotonin Promotes Tumor Growth in Human Hepatocellular Cancer, Hepatology (2010) in press.

2.2.2 Pancreatitis Research Laboratory

Prof. Dr. phil. II
Rolf Graf

Dr. med.
Li-Kang Sun

Dipl. phil. II
Theresia
Reding Graf

Martha Bain

cand. med.
Soo-Young Kim

Pathophysiology of pancreatitis
Reding Graf T, Sun LK, Bain M, Graf R

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.
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.

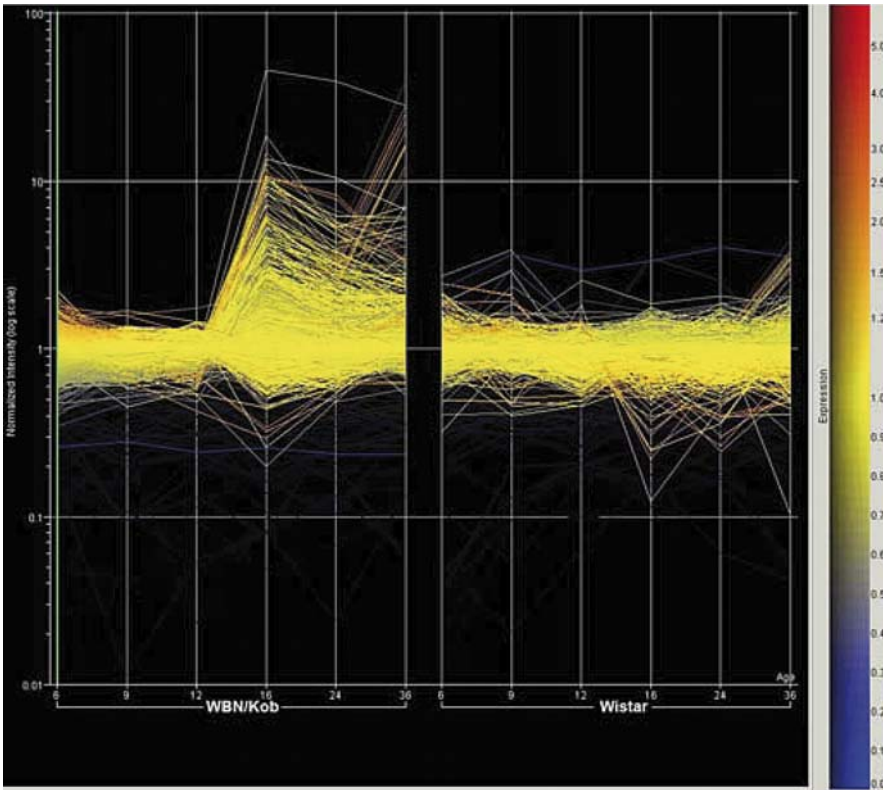


Figure 1: Global gene regulation of genes in the pancreas of WBN/Kob rats (with pancreatitis) and control Wistar rats.

Role of serotonin in disease and regeneration of the pancreas
Silva A, Bain M, Moritz W, Graf **R**

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.
Animals were subjected to an inflammatory cycle or their pancreas was resected. Analysis of expression of transcripts coding for cytokines and growth factors demonstrated that serotonin differentially affects these genes. A conceptual drawing indicates regulatory changes (Figure 2).

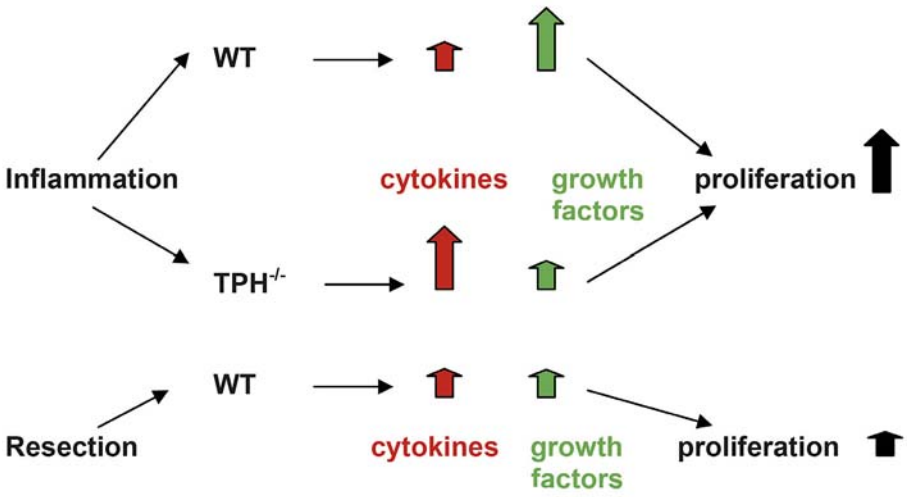


Figure 2: Changes in gene expression depending on whether peripheral serotonin is present. Experiments were conducted to invoke either an inflammatory stimulus or to resect 60% of the pancreas.

Collaborations:

- Dr. Mathias Heikenwälder, Institut für Neuropathologie, Universitätsspital Zürich
- Dr. Achim Weber, Institut für klinische Pathologie, Universitätsspital Zürich
- Dr. Luc Härter, Klinik für Unfallchirurgie, Universitätsspital Zürich
- Dr. Max Bachem, Uniklinikum Ulm, Deutschland

Selected references:

- 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
- Keel M, Härter L, Reding Th, Sun L.-K, Hersberger M, Seifert B, Bimmler D, Graf R (2009) Pancreatic stone protein is highly increased during post-traumatic sepsis and activates neutrophil granulocytes. Critical Care Medicine 37:1642-8.
- Reding Th, Sun L.-K, Hersberger M, Seifert B, Bimmler D, Graf R. Inflammation-dependent expression of SPARC during development of chronic pancreatitis in WBN/Kob rats and a microarray gene expression analysis. Physiological Genomics 2009; 38:196-204

2.2.3 Surgical Skill Center

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

Jérôme Gapany

Hahnloser D, Gapany J

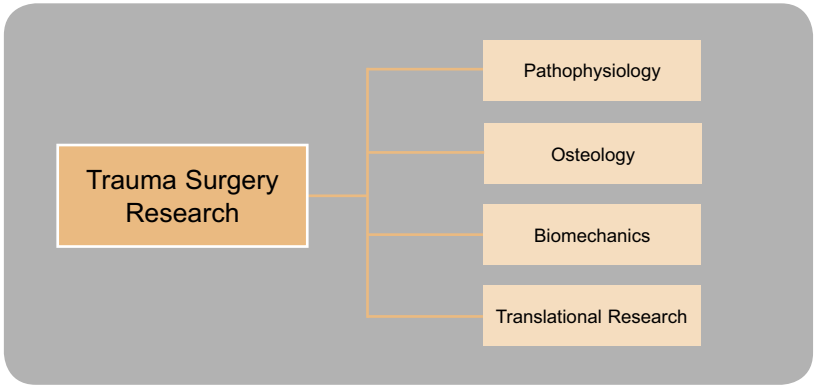
The use of virtual reality (VR) has gained increasing interest to acquire laparoscopic skills outside the operating theatre and thus increasing patients' safety. We valuated 735 surgical trainees from 28 countries at the International Gastrointestinal Surgery Workshop 2006-2008, held in Davos, Switzerland. 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

Prof. Dr. med.
Guido Wanner

PD Dr. med.
C. Werner

Prof. Dr. med.
Hans-Peter Simmen



2.3.1 Pathophysiology

Dr. med.
D. Rittirsch

Prof. Dr. med.
Guido Wanner

Dr. rer. nat.
Luc Härter

S. Hemmi

De novo Synthesis of the complement anaphylatoxins C3a and C5a by Neutrophil Granulocytes
Rittirsch D, Härter L, Hemmi S, Simmen H-P, Wanner G.

The complement system comprises more than 30 plasma proteases, which are activated in a sequential manner. As part of the first line of defense, the complement systems provides direct microbial killing (membrane attack complex, MAC), opsonisation (C3b), as well as release of powerful pro-inflammatory C3a and C5a which exhibiting cytokine-like features.
The complement system plays in important role in many inflammatory diseases, including systemic inflammation and sepsis. In particular, in clinical studies of sepsis, increased concentrations of C3a, C4a and C5a in the plasma have been linked to poor outcome and survival.

Based on preliminary data, we hypothesize that activated neutrophils (PMN) as an important entity of innate immunity de novo-synthesize the complement anaphylatoxins C3a and C5a to locally release them at the site of inflammation (– and not only activate circulating, liver-derived complement proteins by the release of proteases). Therefore, human PMN and/or PBMC are isolated from whole blood by the technique of Ficoll-Hypaque gradient centrifugation (Pharmacia Biotech) and dextran sedimentation, followed by hypotonic lysis of residual RBC. Isolated cells (5 x 10^6 cells/mL) are incubated in HBSS for up to 4 h at 37°C in the presence of LPS (500 ng/ml; serotype O111:B4), BSA IgG immune complexes (IgGIC, 100 mg/ml), or lipoteichoic acid (LTA, 10ug/ml). After incubation the supernatant is collected for assessment of C5a and C3a by ELISA and pellets are lysed with RIPA buffer (Upstate) for immunoprecipitation analyses and westernblot. Preliminary experiments with human PMN from healthy volunteers showed a LPS-induced release of C5a in supernatant (Fig.1 left) and a corresponding reduction in cellbound C5a after 1hour (Fig. 1 right). Further experiments will focus on C3a and C5a production and release from leukocytes from trauma patients.

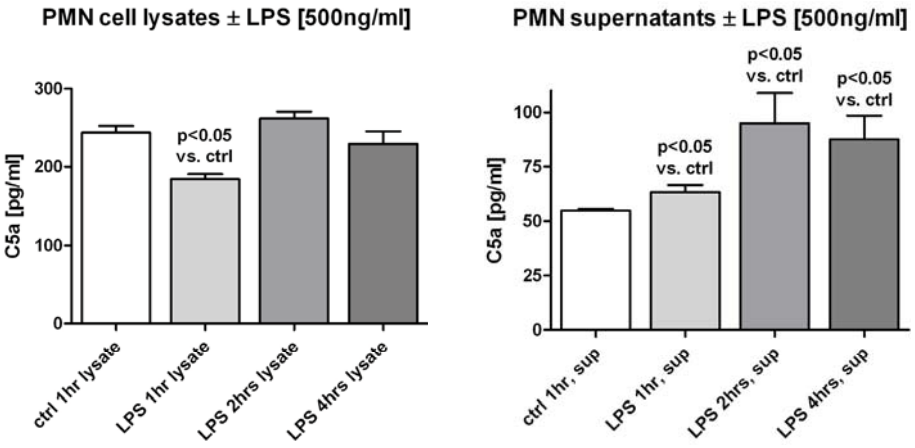


Fig. 1 Detection of C5a in supernatant and celllysate from stimulated human neutrophil granulocytes. Neutrophil granulocytes (PMN) from healthy volunteers were isolated and stimulated with LPS for up to 4hours. Supernatant and celllysates were analysed by a C5a-specific ELISA.

2.3.2 Osteology
Heterotopic Ossification

Dr. med.
SJ. Zimmermann

PD Dr. med.
CML. Werner

Dr. med.
CC. Würgler-Hauri

Dr. med.
M. Scheyerer

New Approaches

Zimmermann SJ, Würgler-Hauri CC, Scheyerer M, Werner CML, Simmen, H-P

Heterotopic ossification of soft tissue is a significantly disabling problem in orthopaedic surgery. Severe joint contracture, ankylosis, spasticity, neurovascular compression, pressure ulcers leading to significant disability may be due to heterotopic ossification. Therapeutical options include NSAID's and local radiation as well as bisphosphonates, all of which inherently carry major disadvantages such as delayed fracture healing and impairing ossification. Hypoxia reportedly stimulates the secretion of HIF-1α. This leads to an increased VEGF production, which acts as a main stimulus for angiogenesis and formation of heterotopic ossification. The inhibition of this pathway could be an essential therapeutical approach. Echinomycin as an antibiotic agent allegedly inhibits the production of VEGF. Therefore we used an established animal model and examined the heterotopic ossification after treatment with Echinomycin. Male CD-1 mice (n=20) were used in this study as approved by the relevant Swiss authorities. All mice underwent bilateral Achilles tendon tenotomy and were divided into groups: Control (n=10), Echinomycin (n=10). The control group underwent Achilles tenotomy only. The Echinomycin group received 10 mcg Echinomycin subcutaneously for 4 weeks, followed by 6 weeks of rest and cage activity only. After 10 weeks the limbs were harvested and Micro CT was performed with a nominal resolution of 30micrometers. Heterotopic bone volume was then identified in 3d images.

Statistical analysis was performed using the Wilcoxon rank sum test. In 12% of the samples no heterotopic ossifications were found. In all other samples, heterotopic ossifications with a bone volume ranging from 0.000 – 1.649 mm3 were found. The mean bone volume in the control group was 0.976mm3 whereas the mean bone volume in the Echinomycin group was 0.092mm3. Range: 0.00 - 0.488 mm3 (p 0.003). Although a significant decrease in bone volume could be achieved, it was not possible to completely prevent the occurrence of heterotopic ossification. Further trials will be done to investigate the clinically disabling problem of heterotopic ossification and its treatment.

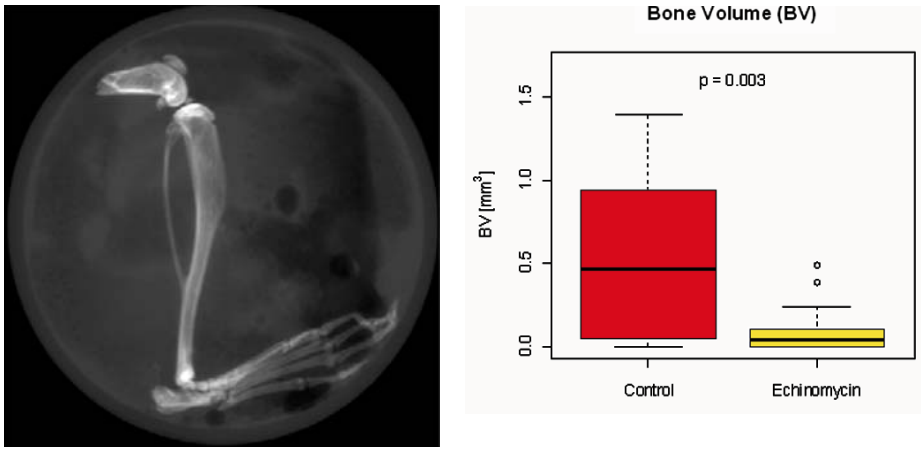


Fig. 2 Effect of Echinomycin treatment on heterotopic bone formation in mice. Representative micro-CT after treatment with Echinomycin (left), and graphic representation of bone volume in untreated controls vs. Echinomycin-treated mice (right). A significant reduction in hind limb bone volume (roughly 90%) could be observed in the group treated with Echinomycin (p= 0.003).

2.3.3 Biomechanics

Dr. med.
G. Osterhoff

PD Dr. med.
CML Werner

Dr. med.
C. Ossendorf

Biomechanical optimisation of plate fixation in proximal humerus fractures
Osterhoff G, Ossendorf C, Werner CML, Simmen H-P

This group has only recently been established.

2.3.4 Translational Research


Dr. med.
V. Schoenborn


Prof. Dr. med.
Guido Wanner


Dr. med.
D. Rittirsch


Dr. med.
E. Wanner


Dr. med.
A. Billeter


Dr. med.
S. Günkel


Dr. med.
M. Turina


Dr. med.
C. Witschier


Dr. med.
V. Neuhaus


Dr. med.
U. Erdmenger

Establishment of a multiplexed transcriptomic marker to improve diagnosis of sepsis and predict the "window of opportunity" in trauma patients
Schoenborn V, Rittirsch D, Billeter A, Günkel S, Simmen H-P, Turina M, Wanner G

Patients after a severe trauma are at high risk to develop post-traumatic complications such as a systemic inflammatory response (SIRS), (multi) organ-dysfunction (MODS) and/or -failure (MOF) and sepsis. The patient's immune responses after trauma is influenced by his genetic background and the environment (Fig.3), and is characterized by a sudden increase of multiple blood mediators also known as a cytokine storm. Many factors involved have been described e.g. IL-1, IL-6, IL-8, TNF, CRP and PCT. However, the underlying mechanisms of this inflammatory response remain obscure. Treatment of patients with severe trauma is performed by two alternative approaches: In "Early total care" all necessary operations are carried out immediately, whereas in the "Damage control" concept the patient is stabilized first and final operations which might present a second stress/trauma for the patient are performed some days (4-7 days) later during the "window of opportunity". However up to date no exact measures exist to precisely define this time point.

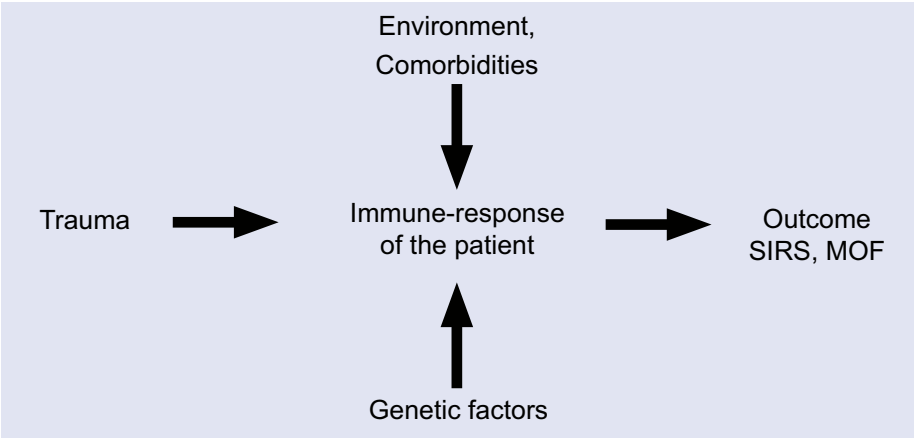


Fig. 3 Factors influencing the patient's response to trauma.

Transcriptomic analyses aim to assess the gene expression patterns related to sepsis of different etiology and in different age groups, in particular as these signatures relate to progression of sepsis. Based on genes identified by microarray analysis we plan to establish an RT-PCR-based test. These marker genes will be confirmed in an independent, prospectively collected patient cohort to assess feasibility of an RT-PCR-based diagnostic test derived from transcriptomic profiling.
A combined cross-sectional and longitudinal approach will enable us to test the hypothesis that multiplexed transcriptomic signatures allow an earlier diagnosis and risk prediction of SIRS related sepsis with better sensitivity and specificity, and enable us to define the optimal time point for the "window of opportunity" more precisely.

Achievements 2009

- Complete restructuring of the research group
- Stem Cell Project with Dr. M. Calcagni, Clinic of Reconstructive Surgery, USZ
- Expression pattern project, Cooperation with Prof. Bauer, Clinic for Anesthesiology and Intensive Care Medicine, Jena, Germany
- Active participation in the DGU “Trauma Datenbank”, Köln, Germany

Collaborations:

- Markus Huber-Lang, Dept. of Trauma Surgery, University Hospital Ulm, Germany
- Peter A. Ward, Dept. of Pathology, University of Michigan, Ann Arbor, USA
- Beatrice Beck-Schimmer, Dept. of Anesthesiology, University Hospital Zurich
- John Stover, Clinic for Intensive Care Medicine, University Hospital, Zürich
- Michael Bauer, Clinic for Anesthesiology and Intensive Care Medicine, University Hospital Jena, Germany
- Biomechanics Laboratories, Orthopedic Research Laboratory, University Hospital Balgrist, Zürich
- E. Neugebauer, „DGU TraumaDatenbank“, Cologne, Germany

Selected references:

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- Pancreatic stone protein is highly increased during posttraumatic sepsis and activates neutrophil granulocytes. Keel M, Härter L, Reding T, Sun LK, Hersberger M, Seifert B, Bimmler D, Graf R. Crit Care Med. 2009;37(5):1642-1648.
- Early expression changes of complement regulatory proteins (CRegs) and C5a receptor (CD88) on leukocytes after multiple injury in humans. Amara U, Kalbitz M, Perl M, Flierl MA, Rittirsch D, Weiss M, Schneider M, Gebhard F, Huber-Lang M. Shock. 2009 Oct 27. [Epub ahead of print]

- Inhibition of complement C5a prevents breakdown of the blood-brain barrier and pituitary dysfunction in experimental sepsis. Flierl MA, Stahel PF, Rittirsch D, Huber-Lang M, Niederbichler AD, Hoesel LM, Touban BM, Morgan SJ, Smith WR, Ward PA, Ipaktchi K. Crit Care. 2009;13(1):R12.
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- Immortalized human skin fibroblast feeder cells support growth and maintenance of both human embryonic and induced pluripotent stem cells. Unger C, Gao S, Cohen M, Jaconi M, Bergstrom R, Holm F, Galan A, Sanchez E, Irion O, Dubuisson JB, Giry-Laterriere M, Salmon P, Simon C, Hovatta O, Feki A. Hum Reprod. 2009;24(10):2567-2581.
- Titanium induced production of chemokines CCL17/TARC and CCL22/MDC in human osteoclasts and osteoblasts Dieter Cadosch, Oliver P. Gautschi, Erwin Chan, Hans-Peter Simmen, Luis Filgueira Journal of Biomedical Materials Research Part A Volume 92, Issue 2, Date: February 2010, Pages: 475-483
- Titanium uptake, induction of RANKL expression, and enhanced proliferation of human T-lymphocytes Dieter Cadosch, Michael Sutanto, Erwin Chan, Amir Mhawi, Oliver P. Gautschi, Brilliana von Katterfeld, Hans-Peter Simmen, Luis Filgueira Journal of Orthopaedic Research Volume 28, Issue 3, Date: March 2010, Pages: 341-347

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

PD Dr. med. Maurizio Calcagni

Prof. Dr. med. Guido Wanner

Dr. sc. nat. Johanna Buschmann

Dr. rer. nat. Luc Härter

PD Dr. med. Nicole Lindenblatt

Dr. sc. nat. Shuping Gao

Manfred Welti

Sonja Hemmi

Development of Bone Grafts Using Adipose Derived Stem Cells and Different Scaffolds: Impact of Pre-engineered Capillaries in Critical Size Grafts
Gao S, Buschmann J, Härter L, Hemmi S, Welti M, Lindenblatt N, Wanner G, Calcagni M, Simmen H-P, Giovanoli P

There is a clinical need for bone grafts in order to treat defect fractures, osteoporosis and osteosarcoma [1]. Bone defects that show no complete osseous regeneration during the lifetime are termed critical size bone defects and require some form of bone substitution. Available bone grafts are very limited with respect to size and 3D architecture, so that often healthy bone has to be removed and used as autograft in bone reconstruction. In our study starting in November 2009, 2 fat samples were included up to date, one from lipoaspirate, and another surgically extracted fat. Isolated ADSC were cultivated in DMEM supplemented with β-FGF (4ng/mL). Flow cytometric analysis of CD11b, CD14, CD15 expression were negative, whereas expression of CD13, CD29, CD44 and CD105 were positive, as indicated by a shift of the specific fluorescence to the right (Figure 1, left). These results were confirmed by immunohistochemistry (Figure 1, right) where cells were also stained with DAPI to visualize the cell nuclei.

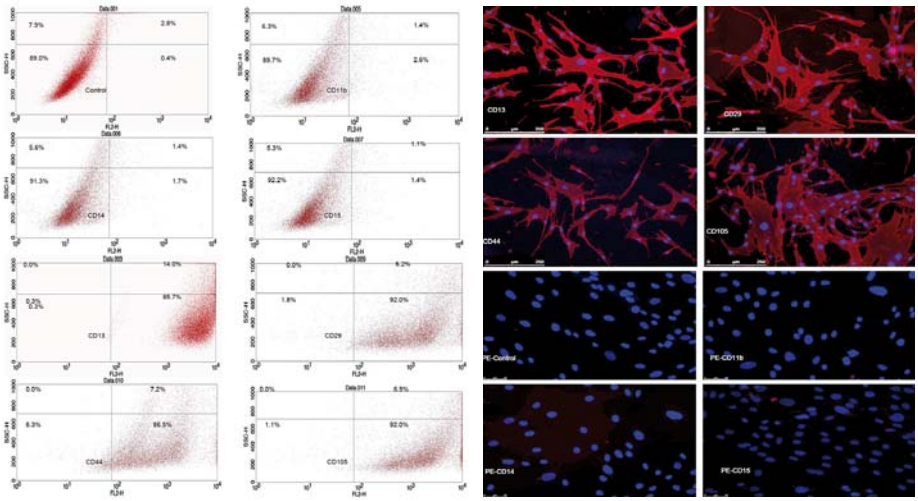

Figure 1. Analysis surface markers on isolated adipose-derived stem cells. Expression of the surface markers (CD11b, CD13, CD14, CD15, CD29, CD44 and CD105) on ADSC was monitored by flowcytometry (left) and immunohistochemistry (right). Cells were stained with Phycoerythrinelabeled antibodies and specific fluorescence monitored in the cytometer (left, FACSCalibur, BD) or microscope (right with DAPI staining).

PCR analysis of RNA expression revealed positive bands for Nanog, BMP6, Oct4, Vimentin, Tert, CD10, CD13, CD44, CD59, CD105, Coll.I, and Coll.V and negative results for Sox2 and CD166 (Fig.2). ADSC differentiated into putative osteoblast cells exhibited an osteoblastlike morphology and expressed several genes consistent with the osteoblast phenotype, confirming differentiation of ADSC into osteoblasts. This was further examined by staining ADSC cultured in osteoblasts differentiation medium for 8, 15, 21, 34 days with Alizarin Red, detecting the presence of calcific deposition by cells of an osteogenic lineage and after “von Kossa” to quantify mineralization. Microscopic examination revealed positive staining typical for osteoblasts (Figure 3).

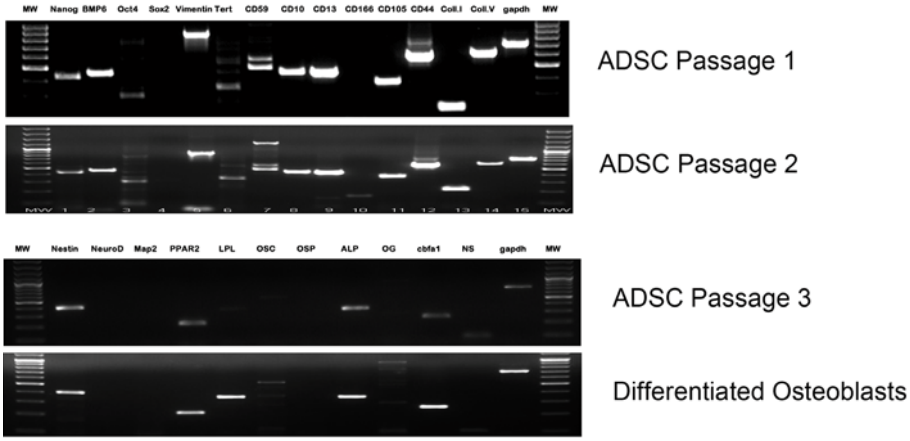

Figure 2. Analysis of mRNA expression from ADSC and differentiated osteoblasts by PCR. Cells were lysed, mRNA was isolated and transcribed to cDNA. PCR with specific primers was performed (30cycles) and PCR products were separated on agarose gels (1.5%). Ethidiumbromide-stained bands were visualized under UV light.

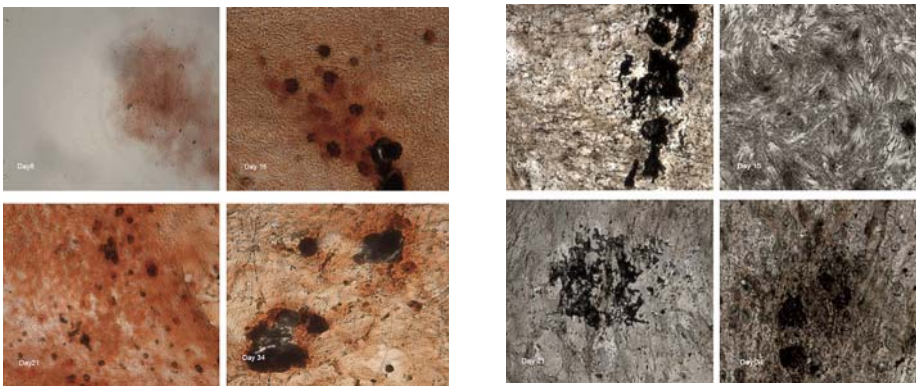

Figure 3. Analysis of ADSC differentiated into osteoblasts. ADSC cultured for 8, 15, 21, 34 days in 4-chamber slides were stained with Alizarin Red (left) or after “von Kossa” (right). Visual examination was performed under the microscope.

The next steps in this project will include further acquisition of fat samples, isolation and differentiation of ADSC into osteoblast and endothelial cells and cultivation of ADSC-derived osteoblasts and endothelial cells on suitable scaffolds to induce vascularisation.

Collaborations:

- Trauma Surgery, University Hospital Zurich
- Reconstructive Surgery, University Hospital Zurich
- Thorax Surgery, University Hospital Zurich
- N. Hild, O. Schneider, W. Stark, ETHZ, Functional Materials Laboratory, Institute for Chemical and Bioengineering

Selected references:

- Giannoudis, P.V., et al., Fracture healing in osteoporotic fractures: Is it really different? A basic science perspective. Injury, International Journal of Care Injured, 2007. 38S1: p. S90-S99.

2.5 Plastic, Hand & Reconstructive Surgery Research

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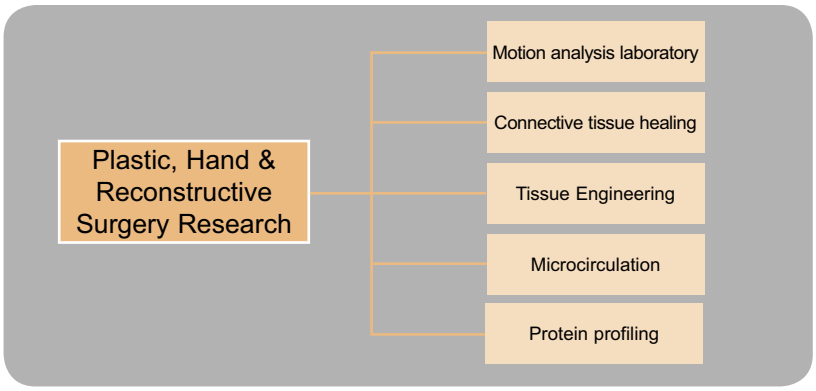

2.5.1 Motion Analysis Laboratory

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Rabbits having one or both hind legs operated: Can we spare animals according to the 3R principle? A motion analysis.
Buschmann J, Müller A, Nicholls F, Calcagni M, Giovanoli P

While planning the experimental design for a new technique of repairing Achilles tendon ruptures in rabbits, we were forced to decide whether only one hind leg per rabbit could be operated while a second rabbit would act as a control - or whether both hind legs would meet our claims (one hind leg operated with a new technique; the other treated by a conventional suture serving as internal control). If the outcome is identical, the second way will be preferred because only half of the number of the rabbits is used (saving animals and costs). We refer to the theoretical framework of the 3R principle, originally proposed by Russell and Burch (Russell, W.M.S., Burch, R.L., 1959. The Principles of Humane Experimental Technique. Universities Federation for Animal Welfare Wheathampstead, England (reprinted in 1992)).

By 2D motion analysis during the 12 week post-op phase, rabbits with both, with one and with none hind legs operated are videotaped, and the range of motion (Figure 1) as well as the hopping length is determined. Three markers are set on the leg by black felt pen after shaving the corresponding spots. During hopping, the angle is having a wide range as shown by Simons et al. [1] (Figure 2).

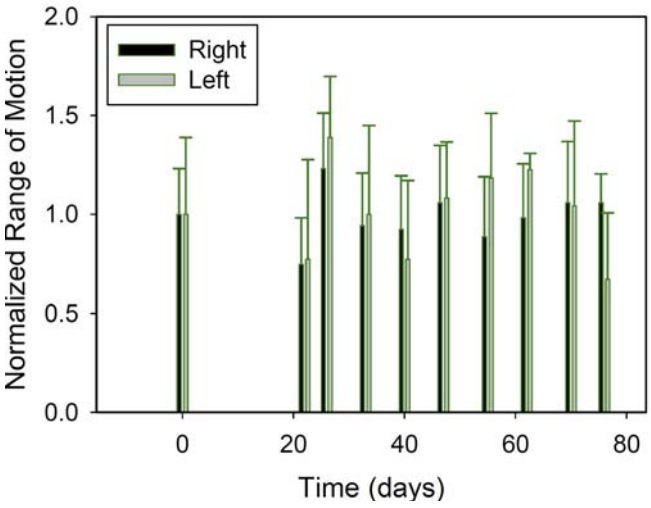

Figure 1: Normalized range of motion as a function of time: day 0 is before operation, followed by 21 days of cast (no data) and weekly motion analysis. Error bars indicate standard deviations (n = 6).

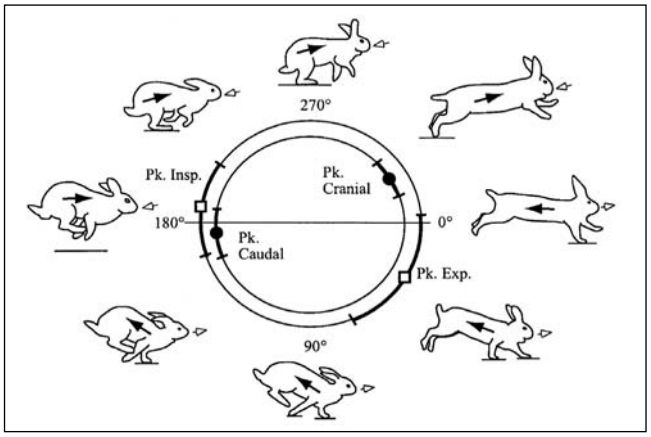

Figure 2: Picture taken from [1] showing the wide range of motion during hopping.

Collaborations:

- Reconstructive Surgery, University Hospital Zurich
- F. Nicholls, Biologisches Zentrallabor (BZL), University Hospital Zurich

Selected references:

- Simons, R.S., *Running, breathing and visceral motion in the domestic rabbit (Oryctolagus cuniculus): Testing visceral displacement hypotheses.* Journal of Experimental Biology, 1999. 202(5): p. 563-577.

2.5.2 Connective Tissue Healing


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Dr. med. Maurizio Calcagni


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Gabriella Meier-Bürgisser


Prof. Dr. med. Pietro Giovanoli

Improvement of tendon repair in hand surgery: Application of a new surgical technique in the rabbit Achilles tendon model in vivo
Buschmann J, Calcagni M, Müller A, Meier-Bürgisser G, Feldmann K, Tervoort T, Neuenschwander P, Bonavoglia E, Fessel G, Snedeker JG, Giovanoli P

There is a need for improving tendon rupture repair in hand surgery. Up to date, various ways of treating tendon ruptures have been studied and applied and modulus, tendon repairs supported by multidisciplinary strategies such as tissue engineering, (bio)scaffolds, stem cells, and/or mechanical stimulation failed to reach normal tendon characteristics [5]. In all studies, the biomechanical properties of the repair tissues were significantly lower than values for normal tissues [6, 7].

In our study here, we apply a new technique for reconstructing tendon ruptures and compare it to conventional techniques (for confidentiality reasons, no details are mentioned). Starting by a pretest with cadaver material, the ultimate force until failure is determined as a baseline value (Figures 1 and 2). Then, using a small number of rabbits, the new surgical procedure is tried out and the healing phase is followed by ultrasonic measurements (thickness of tendon) and motion analysis (range of motion). After a twelve-week post-op time, the biomechanical force until failure and also the tendon histology are compared to the non-treated tendons and to tendons treated by established suture techniques. If improvements are assessed, then the number of rabbits will be enlarged in order to get statistical significance. The study is funded by the Hartmann-Müller Stiftung, the Fonds für Medizinische Forschung and the Wolfermann-Nägeli foundation.

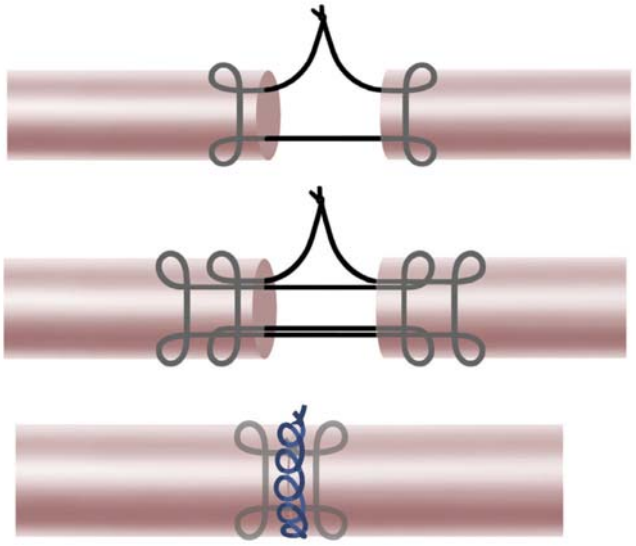

Figure 1: Schema of different conventional suture techniques: 2-strand Kirchmayr suture (top), 4-strand Becker suture (middle) and 2-strand Kirchmayr suture with a running suture (bottom).

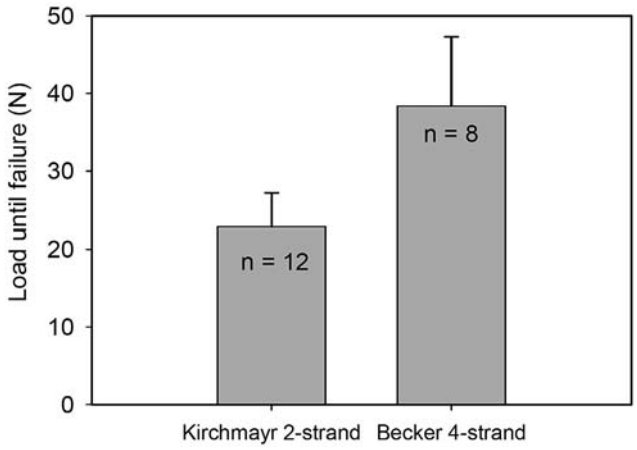

Figure 2: Baseline values of load until failure (N) for conventional suture techniques: 2-strand Kirchmayr (left) and 4-strand Becker (right), both with running sutures.

Collaborations:

- Reconstructive Surgery, University Hospital Zurich
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- P. Neuenschwander, E. Bonavoglia, ab medica, Italy
- G. Fessel, J. G. Snedeker, Balgrist University Hospital, Department of Orthopedics

Selected references:

- Koob, T.J. Biomimetic approaches to tendon repair. in Annual Meeting of the Society-for-Integrative-and-Comparative-Biology. 2002. Anaheim, California: Elsevier Science Inc.
- Huang, D.Q., G. Balian, and A.B. Chhabra, Tendon tissue engineering and gene transfer: The future of surgical treatment. Journal of Hand Surgery-American Volume, 2006. 31A(5): p. 693-704.
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- Riboh, J., et al., Optimization of Flexor Tendon Tissue Engineering With a Cyclic Strain Bioreactor. Journal of Hand Surgery-American Volume, 2008. 33A(8): p. 1388-1396.
- Butler, D.L., et al., Functional tissue engineering for tendon repair: A multidisciplinary strategy using mesenchymal stem cells, bioscaffolds, and mechanical stimulation. Journal of Orthopaedic Research, 2008. 26(1): p. 1-9.
- Awad, H.A., et al., Repair of patellar tendon injuries using a cell-collagen composite. Journal of Orthopaedic Research, 2003. 21(3): p. 420-431.
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2.5.3 Tissue Engineering

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In vitro versus ex vivo histology and high field MRI analyses of preengineered capillary networks in emerging bone grafts: a pilot study

Buschmann J, Steiner R, Welti M, Baltes C, Hemmi S, Giovanoli P, Rudin M, Calcagni M

Repairing large osseous defects is still an unsolved problem. As a standard, autogenous bone grafts are used for the repair. However, there is limited availability, donor site morbidity and insufficient stability. Therefore, critical size tissue engineered bone grafts are needed [1]. Problematic is the insufficient supply of nutrients and oxygen to the centre of such constructs, often ending up in inflammation and cell apoptosis [2].

In our pilot study, we used a polyurethane foam as scaffold and seeded it with human osteoblasts, human endothelial cells or a mixture of both and cultivated it for 1 week in vitro (control: no cells). After that half of all the constructs were gently placed on the chorioallantoic membrane (CAM) of the ex ovo chick embryo and cultivated for another week. At incubation day 15, Gd was either applied i.v. or dripped on top of the CAM samples as well as on the in vitro samples (control: no Gd application). After fixation by formalin, the grafts were collected and (a) analyzed by MRI (ex vivo) in order to quantify the transport of Gd from the chick into the graft and (b) by histology.

In vitro results: Co-cultures of human osteoblasts and endothelial cells showed a better 3D growth into the scaffold material and a higher production of matrix compared to monocultures.

Ex vivo results: Gd transport from the capillary system of the chick embryo into the scaffold (MRI analysis) decreased in the following order: co-culture > control ~ monoculture of endothelial cells > monoculture of osteoblasts.

Histological analyses supported these findings. Whereas monocultures of osteoblasts “closed” the surface of the bone constructs with their calcification of extracellular matrix (hindering vessel ingrowth), the co-culture performed best with respect to perfusion.

A B

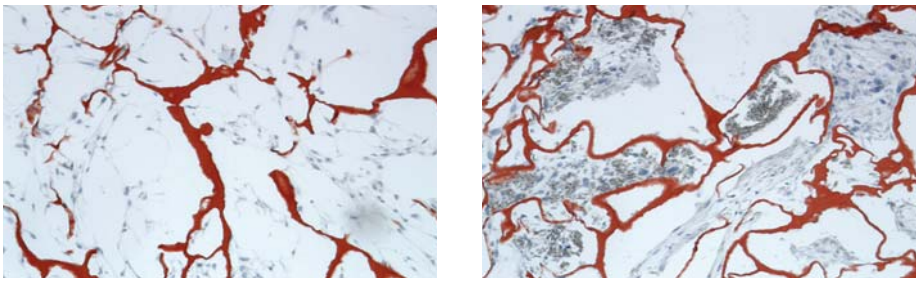

Figure 1: A: monoculture of osteoblasts after 7 days cultivation in vitro and another 7 days on the CAM. B: co-culture of osteoblasts and endothelial cells after 7 days in vitro and 7 days on the CAM. The scaffold is red in this hamalaun/Sudan staining.

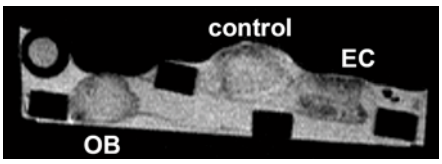

Figure 2: MRI analysis of monocultures only (OB = osteoblasts, EC = endothelial cells) and control (no seeding). The round object in the left upright corner is the reference tube, whereas the black squares are the plastic rings that were used for stabilizing the scaffolds on the CAM membrane.

Collaborations:

- Reconstructive Surgery, University Hospital Zurich
- R. Steiner, Department of Oncology, University Hospital Zurich
- C. Baltes, M. Rudin, Institute for Biomedical Techniques, ETH Zurich, AIC-ETH Hönggerberg
- Trauma Surgery, University Hospital Zurich

Selected references:

- Fuchs, S., et al., Contribution of outgrowth endothelial cells from human peripheral blood on in vivo vascularization of bone tissue engineered constructs based on starch polycaprolactone scaffolds. Biomaterials, 2009. 30(4): p. 526-534.
- Laschke, M.W., et al., Angiogenesis in tissue engineering: Breathing life into constructed tissue substitutes. Tissue Engineering, 2006. 12(8): p. 2093-2104.

Local distinction of perfusion and permeability by an old histological procedure rediscovered for a new target element: Gadolinium - a valiation method for quantitative MRI analysis of tissue engineered constructs

J. Buschmann, W. Angst, R. Steiner, M. Welti, M. Calcagni, P. Giovanoli

Tissue engineered constructs implanted in mice, rats or other animals may be analyzed for their in-situ perfusion capability and their permeability by MRI analysis after i.v. application of a contrast agent such as a Gd complex. Because in a small animal MRI scanner (for example a *Bruker Pharma Scan* 4.7 Tesla scanner) the resolution is as high as 50 µm for reasonable machine times, microvessels perfused with Gd cannot be visualized. The application of a contrast agent i.v. and comparing the sample to the *no-Gd* one, allows only determining quantitatively the sum of perfusion and permeability in the target tissue.

In order to additionally distinguish perfusion from permeability, we rediscovered an old histological procedure developed by Timm in 1936 [1]. The author reports the local precipitation of heavy metals such as iron, silver or gold in the tissues studied. As in 1936 no MRI methods were established yet and no contrast agents such as Gd were used either, the precipitation method was not applied to such an exotic element as Gd in those times. To our best knowledge, this has neither been studied up-to-date.

In the study presented here, we injected Gd-DTPA as a dimeglumin salt i.v. in a chick embryo at incubation day 8.5 of *ex ovo* samples (control 1: no i.v. inject-ion; control 2: some drops onto the chorioallantoic membrane (CAM)).

After fixation by formalin, a piece of the CAM was cut and left in an H_2S-saturated solution overnight according to the procedure by *Timm* [1]. Because the chick embryos were not exposed to any heavy metals during their development, no other heavy metals than Gd were present in measurable concentrations in the tissue. Gd was locally precipitated as Gd_2S_3 (black).

This method can be used for validating the quantitative MRI analysis of the perfusion and permeability of tissues. Moreover, it shows where the contrast agent was at the time the tissue was fixed: whether it circulated in the capillary network or whether there was diffusion into the connective tissue, which gives valuable information while establishing tissue engineered constructs (Figure 1). Further development of this validation method is planned.

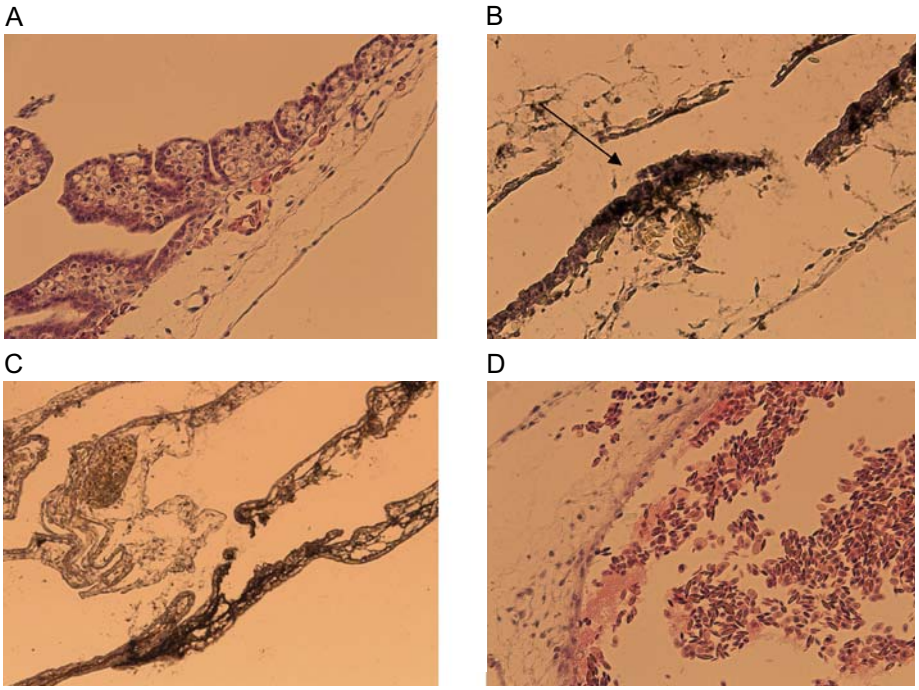

Figure 1 A: i.v. application of Gd-DTPA, no staining for Gd_2S_3; **B:** i.v. application of Gd-DTPA, staining for Gd_2S_3: **C:** drops of Gd-DTPA on CAM, staining for Gd_2S_3; **D:** no Gd-DTPA application, but staining for Gd_2S_3. As can be seen in Figure **B**, Gd was diffusing out of a capillary into the tissue when the CAM was cut (arrow). Figure **C** clearly shows the precipitation of Gd_2S_3 on the CAM surface. *Magnification is 100x.*

Collaborations:

- Reconstructive Surgery, University Hospital Zurich
- W. Angst, Environmental Science, ETH Zurich
- R. Steiner, Department of Oncology, University Hospital Zurich

Selected references:

- Timm, F., *The histochemical proof of "normal" lead in human hard tissue*. Virchows Archiv Für Pathologische Anatomie und Physiologie und Für Klinische Medizin, 1936. 297(3): p. 502-507.

2.5.4 Microcirculation

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Systemic Erythropoietin treatment improves wound healing in microangiopathic mice depleted of apolipoprotein E
Contaldo C, Högger D, Schmidt C, Calcagni M, Lindenblatt N, Giovanoli P

Inadequate blood supply is the most common cause of altered wound healing representing a considerable source of morbidity in daily plastic surgery practice. Erythropoietin (EPO) is intensively investigated for its nonhematopoietic vasculoprotective effects. The present study demonstrates the potential of repetitive systemical EPO treatment to accelerate angiogenesis in hypercholesteremic and microangiopathic murine ischemic wounds. Incisional wounds were created in the mouse dorsal skinfold chamber to monitor revascularization by intravital microscopy on day 1, 3, 5, 7, 9 and 11. We assessed the angiogenetic revascularization of the wound *in vivo* by measuring the functional (neo) vessel density (FVD) after contrast enhancement with 5 % FITC-dextran. The product of red blood cell velocity (RBC) of the newly formed vessels and FVD was taken as an index reflecting the perfusion of the wound with RBCs (RBC perfusion). Hypercholesteremic mice showing established microangiopathy (B6.129P2-ApoE/J) were treated with EPO 1000 IU/kg bw i.p (n=5) or saline (n=5) given at day 1, 5 and 9, and compared to wild type mice (n=5). We used a histological score to assess wound healing and performed immunohistochemical analysis of EPO receptor and eNOS expression on day 3, 7 and 13. On day 9 the hypercholesteremic microangiopathic wounds were characterized by a reduced FVD of 83 ± 5 cm/cm^2 compared to wild type 153 ± 16 cm/cm^2 ($P < 0.01$). EPO treatment increased FVD in microangiopathic wounds to values comparable to that of wild type 163 ± 4 cm/cm^2 ($P < 0.01$ vs. saline treatment). Hypercholesteremic wounds showed 48% less red blood cell perfusion compared to wild type mice on day 9. EPO treatment increased red blood cell perfusion in hypercholesteremic wounds to values similar to that of wild type. The improved wound revascularization was accompanied by a significantly increased amount of EPO receptor (33%) and iNOS protein (55%) at day 7 (both, $P < 0.05$), when compared to saline treated animals. Histological wound score revealed an increased wound healing of 22 ± 1 % at day 7 and 18 ± 3 % at day 13 in treated hypercholesteremic mice (both timepoints $P < 0.01$ vs. saline treated animals). Our data suggests that repetitive systemic EPO treatment improves wound healing in microangiopathic tissue not only by increasing the number of vessels but most importantly by preserving the wound with more red blood cell perfusion.

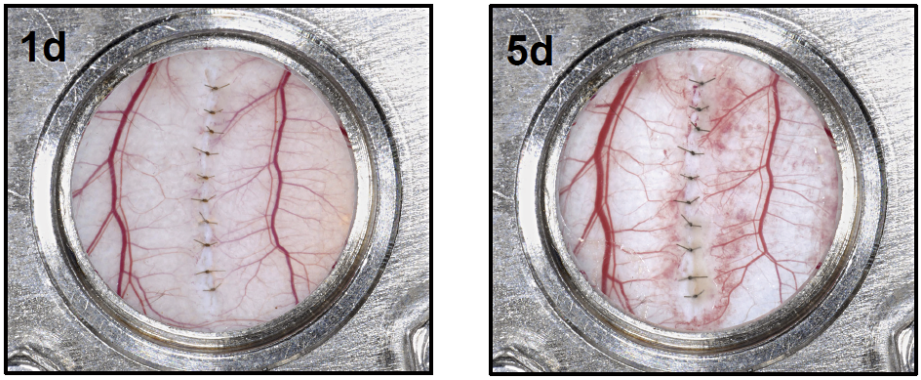
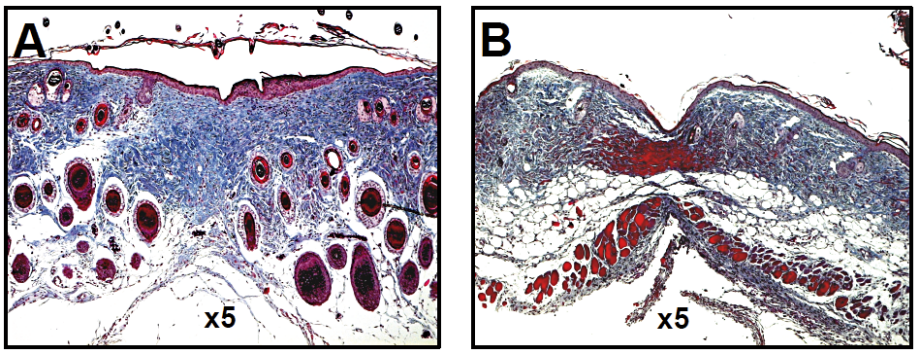


Fig. 1. Incisional wound in the dorsal skinfold chamber.
The murine dorsal skinfold chamber was used for intravital microscopy of the wound. One layer was removed in a 15mm diameter circular area. In the remaining layer consisting of epidermis, subcutaneous tissue, and striated skin muscle an incisional wound was created by a 7mm full-thickness incision, whereafter the wound edges were sutured with 9/0 Nylon sutures in 1mm intervals. The chamber was covered with a glass coverslip, incorporated in one of the titanium frames.



Wild type **ApoE depletion**

Fig. 2. Wound healing abnormalities in mice depleted of apolipoprotein E
Low magnification views of trichrome-stained paraffin sections from the center of 13-day wounds in mice genetically depleted of apolipoprotein E **(B)** and their normal littermates (wild type) **(A)**. Note the well organized deposition of collagen realigned along planes of stress interspersed with a high number of fibroblasts in normal healing wild type mice **(A)**. In contrast, wound healing abnormalities associated with apolipoprotein E depletion is characterized by low amounts of loose collagen matrix with adipose tissue as index of immaturity **(B)**.

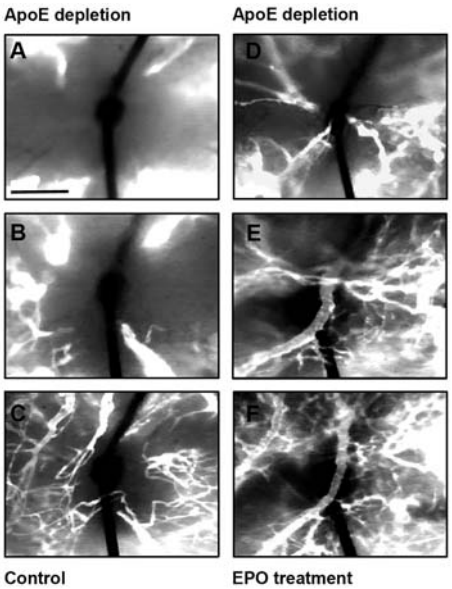


Fig. 3. Intravital fluorescence microscopy [contrast enhancement by 5% FITC-dextran (150,000 mol WT)] in wounds of mice genetically depleted of apolipoprotein E. Representative pictures 3 days **(A,D)**, 5 days **(B,E)** and 9 days (C,F) postwounding are shown (scale bar = 100 µm; 9-0 Nylon suture is shown). Animals were treated with Erythropoietin (EPO) 1000 U/kg body weight intraperitoneally (i.p.) at day 1, 3, 5, 7 and 9 at a concentration of 100 U/mL **(D,E,F)**. Saline treated animals served as controls **(A,B,C)**.

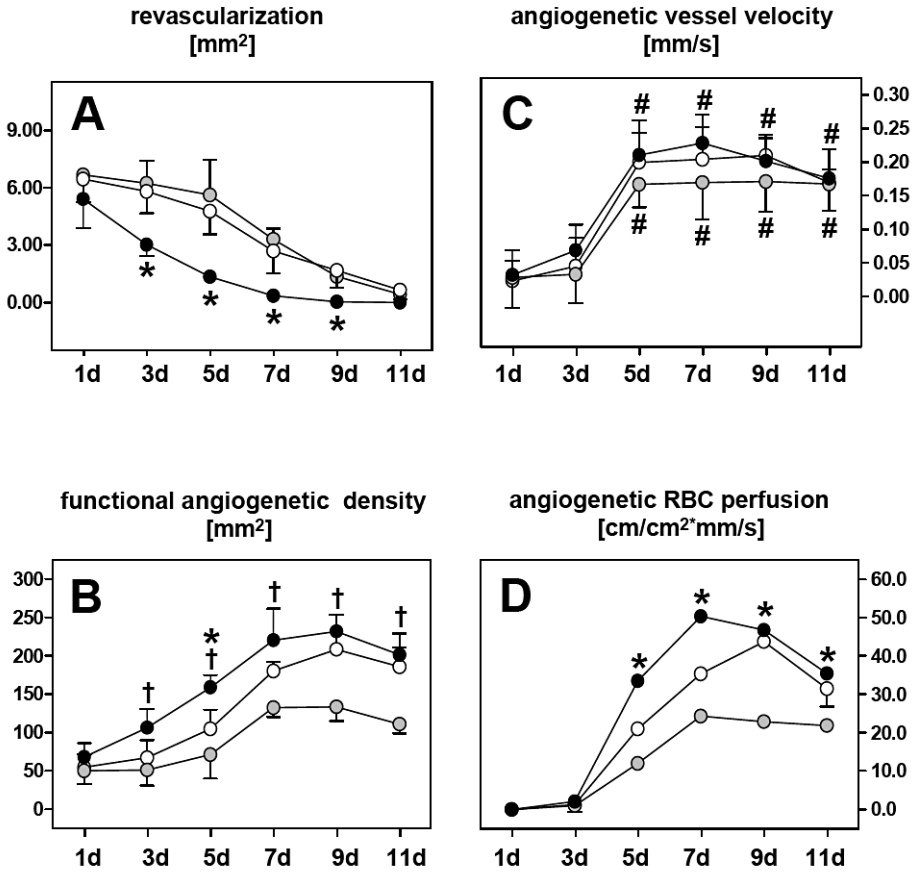


Fig. 4. Ischemic wound revascularization **(A)** was assessed by *in vivo* planimetric measurement (magnification x100, reported in mm^2). Quantitative analysis of microhemodynamics (**B-D**) was measured by intravital microscopy in incisional wounds of wild type mice (○) and ApoE -/- mice with EPO treatment (●) and saline treated controls (◐). * p<0.05, ApoE -/- + EPO vs. other groups; # p<0.05, vs. 1d; † p<0.05, ApoE -/- + EPO vs. ApoE -/- .

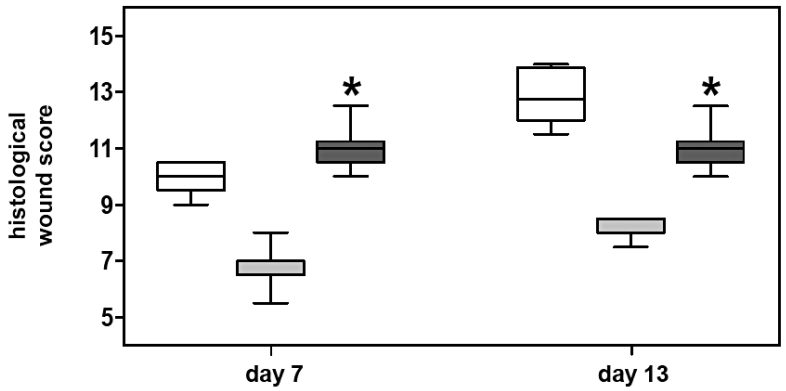


Fig. 5. Histological wound score of normal healing C57B6 mice (white bars) and mice depleted genetically of apolipoprotein E (ApoE -/-) after incisional wounding in the dorsal skinfold chamber. ApoE -/- animals were treated with Erythropoietin (dark grey bars) or saline (grey bars). Total wound score is based on the quality of granulation tissue formation, the number of newly formed capillaries, the cellular invasion and the collagen deposition. * p<0.05, ApoE -/- + EPO vs. ApoE -/-.

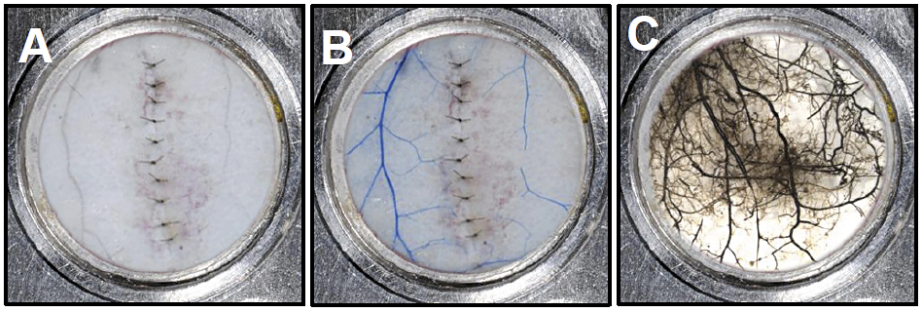


Fig. 6. Vascular corrosion casting. Vascular corrosion casting for micro computed tomography imaging and scanning electron microscopy. The dorsal skinfold chambers were exsanguinated **(A)** and perfused with PU4ii **(B)**. Soft tissue of the skinfold chamber was macerated and dissected from the vascular system after decalcification. Osmium coated samples were subsequently lyophilized. Light microscopic view of a corrosion cast **(C)**.

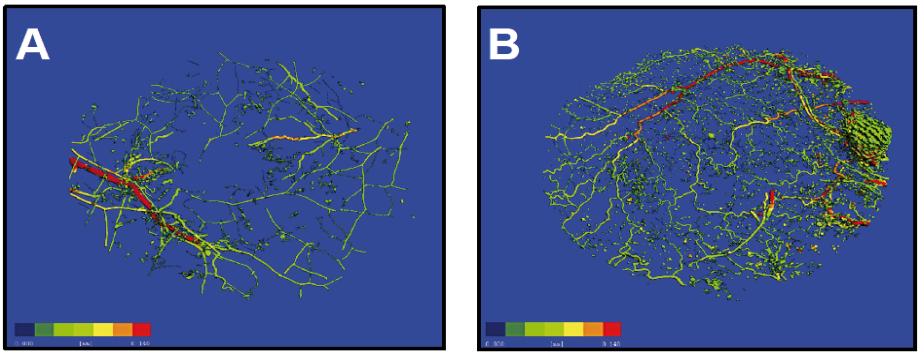


Fig. 7. Micro-CT 3D reconstruction. High resolution Micro-CT reconstruction of PU4ii corrosion casts. Images show dorsal skinfold chambers 5 days after wounding ApoE depleted mice **(A)** compared to their normal healing littermates **(B)**. Colour coding illustrates vessel thickness distribution.

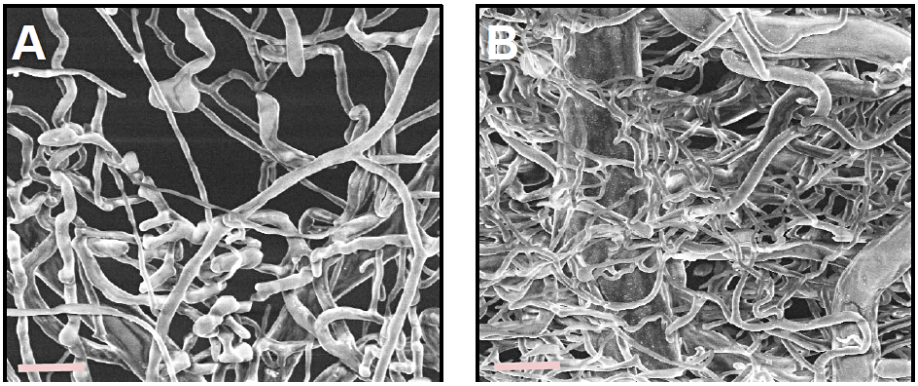


Fig. 8. Scanning electron microscopy. SEM images of vascular PU4ii corrosion casts of mouse dorsal skinfold chambers. Details illustrate the vascular structure within the wound of ApoE depleted mice on day 5 in saline treated controls **(A)** and after EPO treatment **(B)**; (scale bar = 100 µm).

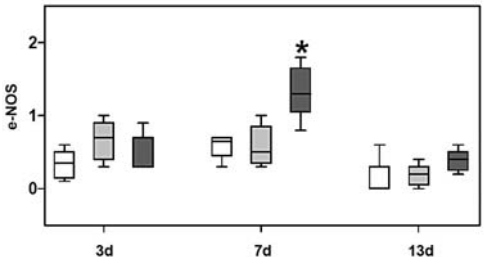


Fig. 9. Semiquantitative analysis of immunohistochemical e-NOS protein expression in normal healing C57B6 mice (white bars) and mice depleted genetically of apolipoprotein E (ApoE -/-) after incisional wounding in the dorsal skinfold chamber. ApoE -/- animals were treated with Erythropoietin (dark grey bars) or saline (grey bars). The intensity of the staining reactions in endothelial cells within the wound was evaluated using a semiquantitative score (graded as 0 = no, 1 = weak, 2 = moderate, and 3 = strong staining. * $p<0.05$, ApoE -/- + EPO vs. ApoE -/- and WT.

Characterization of the vascularization of skin grafts and identification of the vascular mechanisms in a new B6/GFP transgenic *in vivo* mouse model

Lindenblatt N, Calcagni M, Althaus M, Hegland N, Platz U, Contaldo C, Giovanoli P

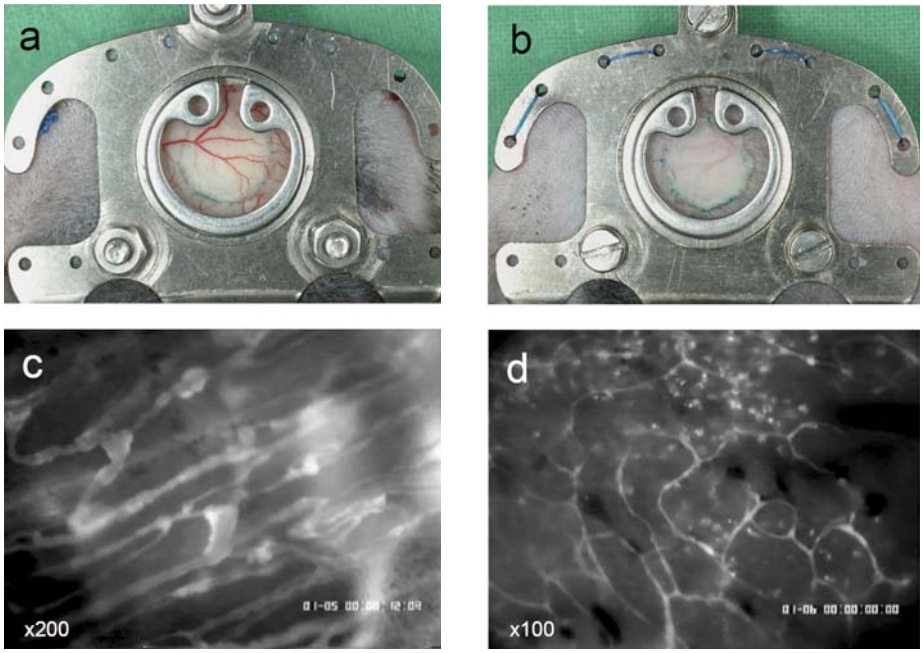
The physiology of skin graft revascularization is still not completely understood until today. However, it has become a standard procedure to achieve closure of skin defects, such as burn injuries and acute or chronic wounds. Lately, the development of tissue-engineered skin substitutes has reanimated the discussion about graft revascularization, because these constructs still lack successful incorporation into the human body. A better knowledge of the mechanisms of the taking of skin grafts and their acquisition of an adequate blood supply would open new perspectives in the manipulation of this process, e.g. by application of substances or materials, and equally facilitate the development of skin substitutes. Numerous efforts of the past to study the revascularization of skin grafts applying histological sections alone yielded very diverse and even contradictory results.

For this purpose we developed a new *in vivo* mouse model, namely the modified dorsal skinfold chamber, allowing for the first time the simultaneous visualisation of the microcirculation wound bed and skin graft over a time period of two weeks. Additionally a crossover design between wildtype C57BL6J und transgenic GFP mice was established allowing for the tracking of GFP (green fluorescent protein) positive cells. By means of this the origination of cells and vascular structure during skin graft revascularization was intended to be identified.
Among the aims of this study were the specific investigation of the transformations within the skin graft in order to gain applicable knowledge on how vascular development during engraftment occurs.
Graft reperfusion started after 72 hours. At 96 hours the formation of spherical protrusions was seen at the graft capillary divisions most likely representing a temporary angiogenic reaction, since confocal microscopy revealed the simultaneous expression of CD31 and desmin. The B6/GFP model confirmed the origination from the autochthonous graft vasculature. Additionally GFP-positive vessels were detected growing in from the wound bed and orientating along the existing vascular structures of the graft. Corrosion casting and evaluation by SEM confirmed the three-dimensional formation of capillaries in the wound bed connecting to the capillary loops of the graft.

These *in vivo* data indicate the connection of angiogenic bed vessels to the existing graft vasculature resulting in reperfusion of the graft. Subsequently wound bed vessels appear to grow long the existing vascular structures within the graft. Additionally we observed a temporary angiogenic response within the capillaries of the skin graft. This novel finding most likely represents a reaction to reperfusion allowing the supply of pro-angiogenic factors to the hypoxic skin graft.

Modified dorsal skinfold chamber

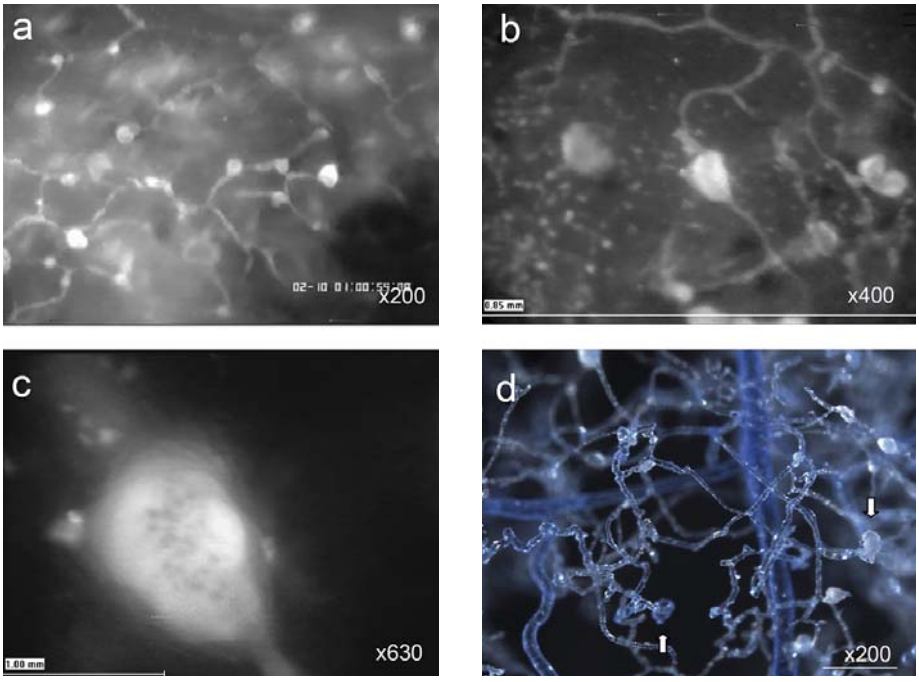
Lindenblatt et al., Plast Reconstr Surg. 2008; 122: 1669-80.



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).

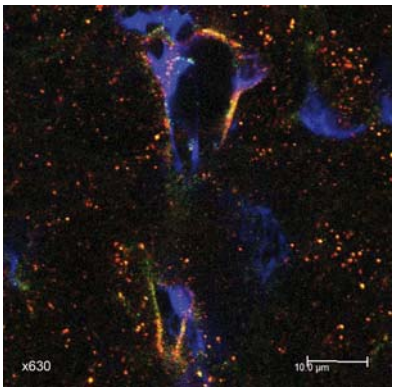
Temporary angiogenic response

Lindenblatt et al., Plast ReconstrSurg. 2009 (accepted).



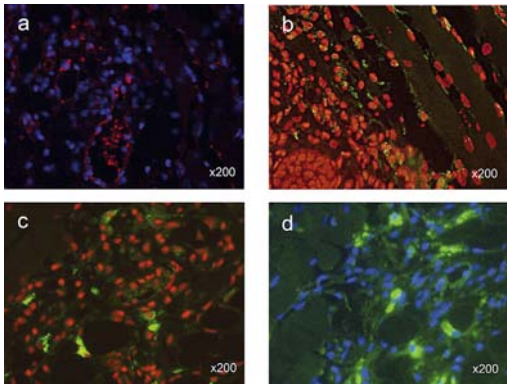
(a) Intravital microscopy revealed the formation of spheric protrusions of the graft capillaries after reperfusion(96 hours). (b,c) At a higher magnifications these bud-like structures appeared to contain cellular material und were up to 10-fold as wide as normal capillaries. Blood flow was slow, but maintained. (d) Light microscopic evaluation of corrosion casts confirmed these vascular structures (arrows).

Angiogenic bud



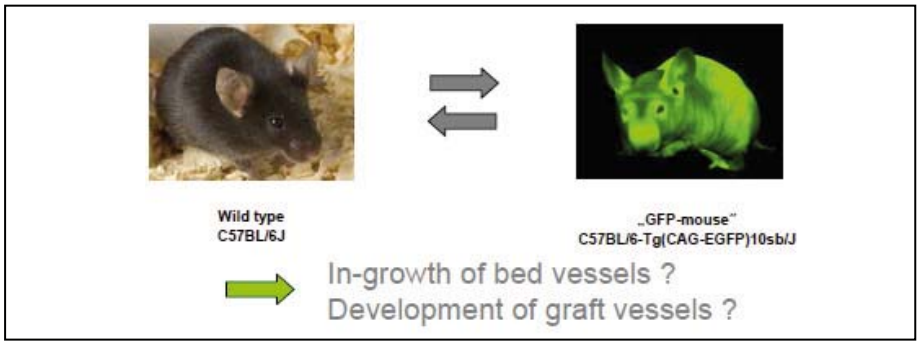
Fluorescent immunohistochemistry and evaluation by confocalmicroscopy showed that the vascular wall of the spherical structures expressed CD31 (green) and the pericyte-marker desmin(red) resulting in yellow overlay. Therefore these structures most likely represent mature angiogenic buds.

Angiogenic factors

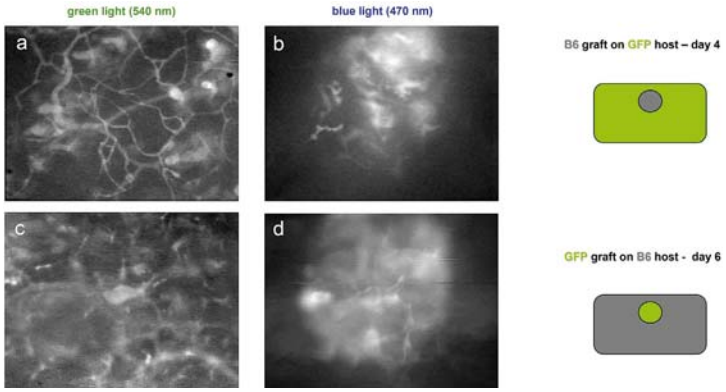


Histological evaluation of the expression of angiogenic factors within the wound bed and the skin graft revealed the up-regulation of Ang-1 (a), the VEGF receptors Flt-1 (b) and Flk-1 (c) as well as of bFGF(d).

Skin grafting cross-over B6/GFP



Vessel origination



(a) Reperfusion of the B6 graft capillaries at day 4 after injection of rhodamine-dextran(MW 150000) and exposition to green light. (b) Evaluation under blue light reveals the presence of green-fluorescent cells within the reperfused graft capillaries suggesting the in-growth of cells from the GFP host bed into the B6 graft. (c) In the opposite case-GFP graft on B6 host-exposition to green light after injection of rhodamine-dextran reveals the formation of angiogenic structures of the graft vasculature. (d) Under blue light it can be seen that this angiogenic response originates from the autochtonous GFP graft vasculature.

Achievements 2009

Talks and Posters

- Contaldo C, Högger D, Lindenblatt N, Calcagni M, Giovanoli P. Systemic Erythropoietin treatment improves wound healing in microangiopathic mice depleted of apolipoprotein E. 8th Day of Clinical Research. Zurich, 16.04.2009.
- Contaldo C, Waygood M, Calcagni M, Giovanoli P. Dose-dependant microvascular response following extracorporeal shock wave application in the striated muscle of the mouse dorsal skinfold. European Plastic Surgery Research Council (EPSRC). Hamburg, 22.08.2009.
- Contaldo C, Platz U, Högger D, Lindenblatt N, Calcagni M, Giovanoli P. Platelet derived serotonin plays a critical role during skeletal muscle ischemia and reperfusion injury. European Congress of Scientists and Plastic Surgeons (ECSAPS). Rotterdam, 18.09.2009.
- Dr. Claudio Contaldo, invited chairperson in the Wound Healing session of the European Congress of Scientists and Plastic Surgeons (ECSAPS). Rotterdam, 18.09.2009.
- Contaldo C. Die verbrannte Hand. 5. Workshop Verbrennungschirurgie. Zurich, 07.07.2009.
- Lindenblatt N, Calcagni M, Platz U, Schmidt C, Contaldo C, Vollmar B, Giovanoli P. Visualisation of skin graft revascularisation in the modified dorsal skinfold chamber - new insights into the processes at the vascular interface. European Plastic Surgery Research Council (EPSRC). Hamburg, 21.08.2009.
- Lindenblatt N, Calcagni M, Platz U, Schmidt CA, Contaldo C, Giovanoli P. Temporary angiogenic response of the graft capillaries and early HIF1-alpha-mediated angiogenesis: new insights into the processes at the vascular interface during skin graft revascularisation. 45th Congress of the Swiss Society for Plastic, Reconstructive and Aesthetic Surgery (SGPRAC). Interlaken, 03.10.2009.
- Lindenblatt N, Calcagni M, Platz U, Schmidt CA, Contaldo C, Vollmar B, Giovanoli P. Novel insights into the process of skin graft revascularization – in-growing wound bed vessels induce a temporary angiogenic response in the graft vasculature. Poster presentation. Meeting of the Society for Microcirculation and Vascular Biology (GfMvB) and the Swiss Society for Microcirculation (SSM). Bern, 09.10.2009.
- Lindenblatt N, Calcagni M, Platz U, Schmidt CA, Contaldo C, Vollmar B, Giovanoli P. Skin graft revascularization involves early hypoxia-mediated angiogenesis within the wound bed, vessel-outgrowth and temporary responses within the graft. Poster presentation. 8th Day of Clinical Research. Zurich, 16.04.2009.
- Högger D, Contaldo C, Calcagni M, Giovanoli P. Systemic Erythropoietin treatment improves wound healing in microangiopathic mice depleted of apolipoprotein E. European Plastic Surgery Research Council (EPSRC). Hamburg, 21.08.2009.

- Högger D, Contaldo C, Platz U, Stotz M, Lindenblatt N, Calcagni M, Giovanoli P. Low-energy extracorporeal shock wave application optimizes murine incisional wound healing. European Congress of Scientists and Plastic Surgeons (ECSAPS). Rotterdam, 18.09.2009.

Collaborations:

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- Prof. Dr. med. Simon P. Hoerstrup, Department of Surgical Research and Clinic for Cardiovascular Surgery, University Hospital Zurich
- Dr. Eric P. Meyer und Alexandra Ulmann, Institute of Zoology, University of Zürich
- Klaus Marquardt, Center for Microscopy and Image Analysis, University of Zürich
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- PD Dr. med. Ernst Reichmann, Tissue Biology Research Unit, Children's Hospital Zurich
- Dr. med. Christian A. Schmidt, Clinic for Cardiovascular Surgery, University Hospital Zurich

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- Tan Q, El-Badry AM, Contaldo C, Steiner R, Hillinger S, Welti M, Hilbe M, Spahn DR, Jaussi R, Higuera G, van Blitterswijk CA, Luo Q, Weder W. The effect of perfluorocarbon-based artificial oxygen carriers on tissue-engineered trachea. Tissue Eng Part A. 2009; 15: 2471-2480.

2.5.5 Protein Profiling

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Protein profiling in plasma proteins

Finke J, Glocker M, Calcagni M, Giovanoli P

The proteome describes the equivalent of the genome at protein level. The proteome is the entirety of proteins of an organism, a cell or a body fluid at a specific time point under specific conditions. This pattern of proteins reflects the present metabolic status which is dependent on the genome, environmental factors, and complex interactions of biomolecules.
Proteome analysis enables a profiling of all peptides and proteins in body fluids that is characteristic for specific conditions and diseases.
The aim of our study is to generate plasma protein profiles in free flap transfer to investigate changes of protein profiles during ischemia and reperfusion. Currently, only clinical examination of the transplanted tissue (e.g. flow, turgor, temperature) gain information about the actual status of the flap. Neither reliable preclinical recognition nor effective prevention measures for partial or complete flap loss are available. The diagnostic criteria used for flap monitoring are variable and inconsistent.
To gain further insight in the qualification of protein profiles for flap monitoring we study protein profiles during transfer of muscle flaps, fasciocutaneous flaps, and bone flaps. We expect to find differences in protein profiles and we will then correlate the findings with the clinical outcome.
Three groups, 30 patients per group, of free flap transfer were considered: muscle flaps, fasciocutaneous flaps, and bone flaps.

Samples are provided (i) by the Division of Oral and Cranio-Maxillofacial Surgery, (ii) by the Division of Otorhinolaryngology, and (iii) by the Division of Plastic, Reconstructive Surgery and Hand of the University Hospital Zurich.
Blood samples are taken intraoperatively prior to arterial anastomosis (t1) from the arterial flap inflow and after arterial anastomosis (t2) from the venous flap outflow to investigate protein changes during time of ischemia and reperfusion in the flap tissue. The control samples are taken from the arterial and venous vessels on the donor side to investigate differences between arterial and venous blood in general.

This study is based on a previous study of the principal investigator [1,2].
A specific plasma protein profile for the HELLP syndrome was generated involving protein areas that contain inter-alpha-trypsin inhibitor heavy chain H4, kininogen 1, fibrinogen gamma chain, transthyretin, haptoglobins, and serum amyloid A with statistically significant expression differences when compared to controls (see figure 1,2). The most striking difference was serum amyloid A (SAA) which was validated and quantitatively assayed by ELISA measurements against human SAA in plasma (see figure 3). Our results show that significant differences in SAA expressions between healthy controls and HELLP patients were obtained, that could function as a part of a marker set for the HELLP syndrome.

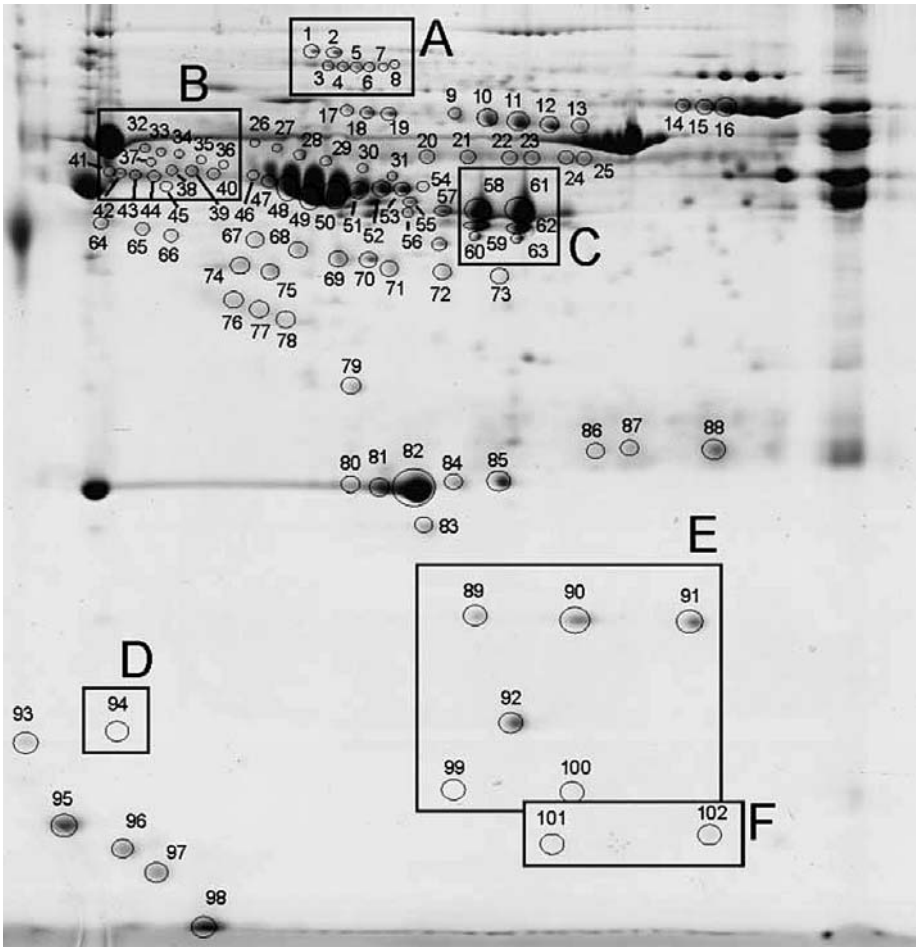

Figure 1: Reference gel for HELLP that presents all identified protein spots and integrates all the changes of plasma protein abundances comparing HELLP patients prior to and after delivery. 2D gel (pH 4-7), stained with Colloidal Coomassie Blue. Boxes A-F show areas with protein expression differences (cf. figure 2).

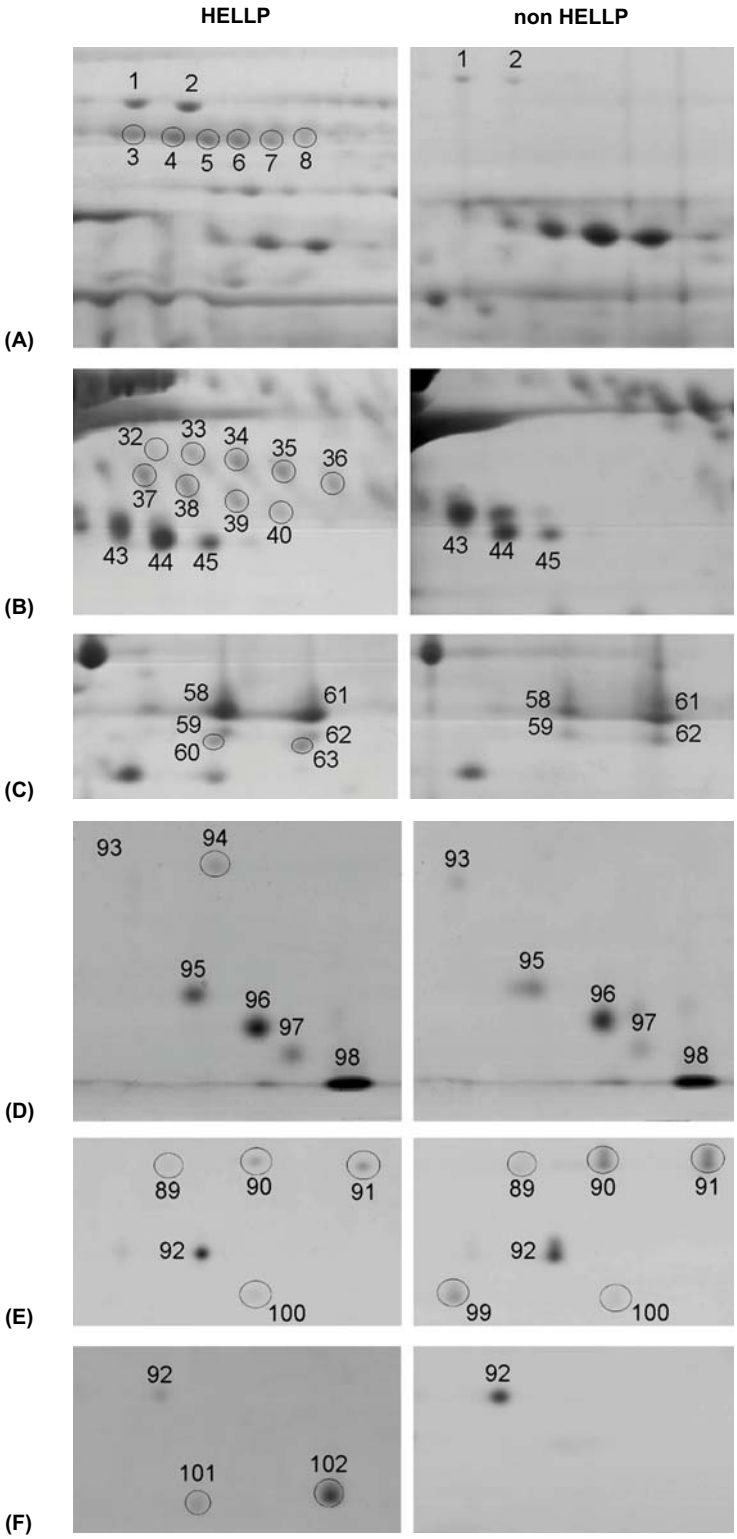

Figure 2: Gel areas A-F present differentially expressed proteins in plasma of HELLP patients as compared to controls. Protein spots with differential abundance are circled. Numbers are identical to those in figure 1.

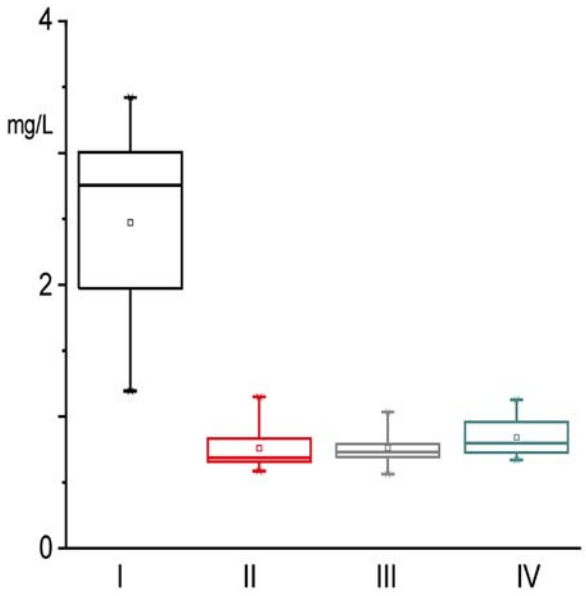

Figure 3: Serum amyloid A ELISA data represent-ed as box and whiskers plots. A significant ($p < 0.001$) increase of SAA levels was found in (I) the HELLP group (n = 18 measurements) compared to the control groups of (II) postHELLP mothers (n = 14 measurements), (III) healthy pregnant women (n = 20 measure-ments), and (IV) healthy mothers / postPregnant controls (n = 12 measurements). All readings were performed in duplicate. Boxes represent the 25^{th}–75^{th} percentiles. The horizontal line within the boxes represents the 50^{th} percentile. Whiskers represent the 5^{th} and 95^{th} percentiles. The small box represent the mean value. The min / max values are depicted as minus (-) signs and the 99^{th} and 1^{st} percentiles as crosses (x).

Collaborations:

- Prof. Dr. rer. nat. Michael O. Glocker , Leiter des Proteom-Zentrums der Universität Rostock, Deutschland
- Prof. Ralph Schlapbach, Managing Director, Functional Genomics Center Zurich, UZH/ETH Zurich, Switzerland
- Dr. med. M. Bredell, Division of Oral and Cranio-Maxillofacial Surgery of the University Hospital Zurich, Switzerland
- Dr. med. G. Huber, Division of Otorhinolaryngology of the University Hospital Zurich, Switzerland

Selected references:

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- Heitner (Finke) JC, Koy C, Kreutzer M, Gerber B, Reimer T, Glocker MO. Differentiation of HELLP patients from healthy pregnant women by pro-teome analysis – On the way towards a clinical marker set. J Chrom B 840(2006): 10-19.

2.6 Thoracic Surgery Research


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Sven Hillinger


Prof. Dr. med.
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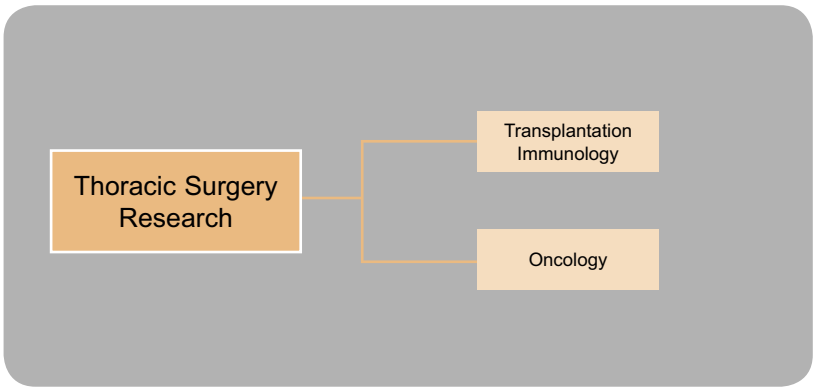

2.6.1 Transplantation Immunology


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Prof. Dr. med.
Stephan Korom

Inhibition of CD26/DPP IV attenuates ischemia/reperfusion injury in a mouse model of orthotopic lung transplantation
Jungraithmayr W, Korom S

The T cell activation Ag CD26/Dipeptidylpeptidase IV (DPP IV) combines costimulatory and enzymatic properties. Catalytically, it functions as an exopeptidase, modulating biological activity of key chemokines and peptides. Here we investigated the effect of organ-specific inhibition of DPP IV catalytic activity on ischemia/reperfusion injury after extended ischemia in the mouse model of orthotopic single lung transplantation. C57BL/6 mice were syngeneic transplanted, grafts were perfused and stored in Perfadex with (treated) or without (control) a DPP IV enzymatic activity inhibitor (AB192). Transplantation was performed after 18h cold ischemia time; following 2h-reperfusion, grafts were analyzed for oxygenation, thiobarbituric acid-reactive substances, histomorphology, immunohistochemistry was performed for leukocyte Ag 6, myeloperoxidase, hemoxygenase 1, vasoactive intestinal protein (VIP), and real time PCR for VIP. Treatment with the DPP IV inhibitor AB192 resulted in significant improvement of gas exchange, reduced lipid oxidation, preservation of parenchymal ultrastructure, reduced neutrophil infiltration, reduced myeloperoxidase expression, increased hemoxygenase 1 expression, pronounced expression of vasoactive intestinal peptide (VIP) in alveolar macrophages and increased mRNA expression of VIP. Inhibition of intragraft DPP IV catalytic activity with AB192 strikingly ameliorates ischemia/reperfusion injury after extended ischemia. Furthermore, preservation of endogenous intragraft VIP levels correlate with maintaining lung function and structural integrity.

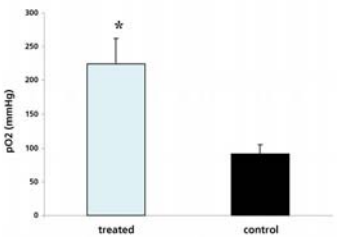


Figure 1 Oxygenation in syngrafts 2h after reperfusion, treated (DPP IV-inhibited) vs. control (Perfadex perfusion only, control) (treated: 224.5 ± 37.6 mmHg vs. control: 91.8 ± 13.6; *P = .002). Values are expressed as mean ± SD

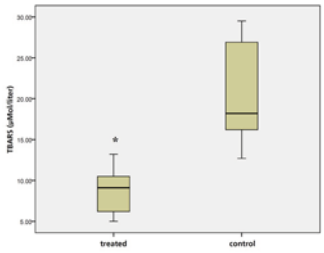


Figure 2: Transplant oxidative stress, comparing controls to DPP IV-inhibited grafts 2h after reperfusion (control: 20.3 ± 6.5 µMol/liter vs. treated: 8.6 ± 3.0; *P = .004).

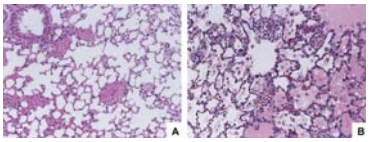
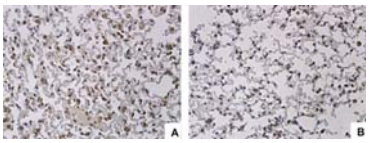


Figure 3: DPP IV-inhibited grafts (**A**) display less edema and macrophages than Perfadex-perfused grafts (**B**) (Orginal magnification: x100; H&E).



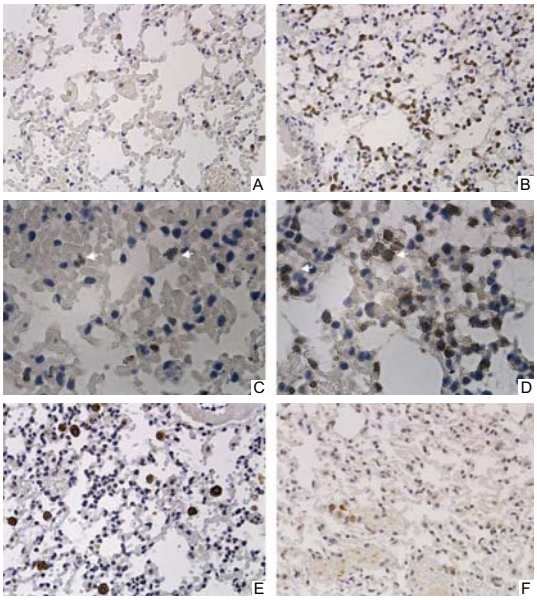


Figure 4: Immunohistochemistry for Ly-6G (A, B; Original magnification: ×400), MPO (C, D; Original magnification: ×400) and HO-1 (E, F; Original magnification: ×200). Comparison of sections of pulmonary parenchyma from DPP IV-inhibited grafts and from Perfadex-perfused grafts. One representative section for each of five grafts is shown. **A**, Sections from DPP IV-inhibited grafts, 2h after reperfusion, displayed reduced numbers of neutrophils (18.9 ± 7.7 positive cells/0.1 mm2) vs. controls, **B** (66.8 ± 11.6 positive cells/0.1 mm2). **C**, Sections from DPP IV-inhibited grafts, 2h after reperfusion, showed infrequent numbers of neutrophils with cytoplasmic granular MPO staining pattern (white arrows) (11.1 ± 3.9 positive cells/0.1 mm2) vs. controls, **D** (47.4 ± 9.0 positive cells/0.1 mm2), whereas grafts perfused with Perfadex only showed multiple neutrophils with cytoplasmic granular MPO expression with a distinct multilobed nucleus. **E**, Sections from DPP IV-inhibited grafts, 2h after reperfusion, showed increased numbers of cells expressing HO-1 (71.6 ± 9.8 positive cells/0.1 mm2) vs. few cells in controls, **F** (19.5 ± 6.1 positive cells/0.1 mm2).

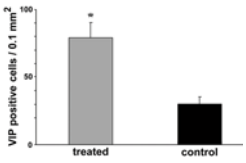


Figure 5: Graft VIP immunohistochemical staining of MΦ. Comparing grafts flushed/stored in Perfadex + AB192 vs. control lungs, flushed/stored in Perfadex with 18 hrs ischemia time and 2 hrs reperfusion. One representative section for each of five grafts is shown. (Original magnification: ×200). **A**, in pulmonary grafts flushed/stored in Perfadex + AB192, staining for VIP was enhanced in MΦ (79.1 ± 11.2). **B**, in pulmonary grafts flushed/stored in Perfadex, there was only moderate staining in MΦ for VIP (30.1 ± 5.6).

Tolerance induction via NK cell mediated elimination of donor antigen presenting cells in mouse lung transplants

Jungraithmayr W, Münz C

Background

Natural killer (NK) cells are a major group of lymphocytes of the innate immune system and important cells in the control of infections and tumors. The activity of NK cells is well controlled by surface receptors which deliver stimulatory but also inhibitory signals, specific for stress-induced self-ligands and major histocompatibility complex (MHC) class I molecules, respectively. Inhibitory receptors, recognizing classical and non-classical MHC class I molecules, prevent NK cells from damaging self-tissues, but allow cytotoxicity towards cells mismatched for MHC class I molecules.

With respect to transplantation, both beneficial and detrimental effects of NK cells have been reported. NK cells are activated after solid organ transplantation and promote killing and cytokine release. In allogeneic bone marrow transplantation, NK cells function as potent effector cells, but also in solid organ transplantation, when NK cells contribute to organ rejection independent of its activating receptors Ly49D and NKG2D, or when developing cardiac vasculopathy upon NK activation. NK cells were shown to promote transplant tolerance to islet allografts through the perforin dependent pathway, and the inhibition of NK-receptor-bearing cells combined with CD28-costimulation blockade established long-term graft acceptance in cardiac allografts. However, NK cells from mice and humans show minimal effector functions when triggered via their stimulatory receptors, suggesting that resting NK cells require additional signals for their activation. In vitro studies have focused on a potential role of myeloid cells for the activation of NK cells and demonstrated that TLR-stimulated bone marrow (BM) - or monocyte derived dendritic cells (DCs) contribute to NK cell activation. Cytokines and NK cell receptor-ligand interactions have also been implicated in the in vitro activation of NK cells by BM- or monocyte-derived DCs. Among those cytokines, IL-15 was shown to be important for the survival, proliferation and the effector function of NK cells when co-cultured with DCs. *In vitro*, the DC-expressed IL-15 receptor α chain-trans presenting IL-15 was responsible for both killing and for IFN-γ production by NK cells. IL-15 mediated activation of NK cells is therefore considered to have a beneficial effect on transplant acceptance by NK cell mediated purging of transplant associated allogeneic DCs.

Large numbers of graft derived donor antigen presenting cells (APCs) contribute significantly to the rejection process either through the direct or the indirect antigen presenting pathway. Early after transplantation, donor DCs present allo-antigen to host lymphocytes, activate and induce the priming of a large quantity of alloreactive T cells for the rejection respons. These strong effector cells migrate into the graft and initiate an inflammation process that eventually results in allograft rejection. The function and survival of a pulmonary allograft decisively depends on the grade and severity of these infiltrating T cells. Via recognition of allogeneic DCs, host NK cells are capable to influence the magnitude of allospecific $CD4^+$ T cell responses and Th cytokine development. In a model of skin transplantation it was shown that host NK cells were able to kill graft derived APCs from skin allografts and induce skin allograft survival, preventing priming of alloreactive T cells in lymphoid and nonlymphoid tissues. Conclusively, it would be beneficial to avoid the infiltration of alloreactive T cells and all of its inflammatory correlates within the pulmonary graft by eliminating allogeneic DCs, which are involved in the priming of this alloreactive response, via NK cell cytotoxicity.

Hypothesis

We hypothesize that upon IL-15 stimulation, host NK cells regulate the life and death of donor-derived APCs, thereby controlling the adaptive T cell response in orthotopic mouse lung transplants. This interaction constitutes a critical step in the acquisition of transplant tolerance.

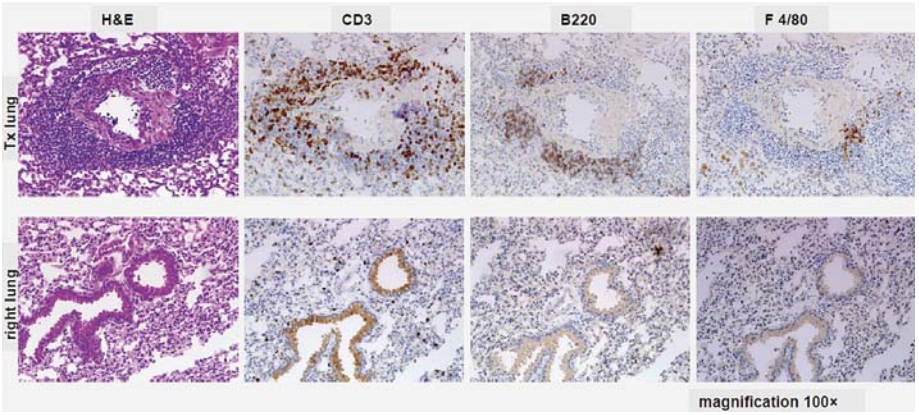


Abb. 1: Histology (H&E) and Immunohistochemistry of T cells (CD3), B cells (B220) and Macrophages (F 4/80) in the transplanted (Tx) and the right, non-transplanted lung at day 5 post transplantation.

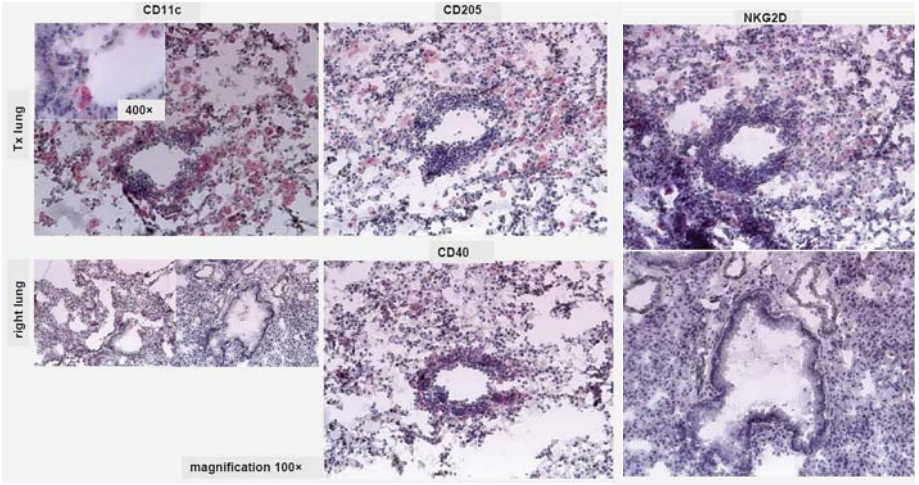


Abb. 2: Immunohistochemistry of dendritic cells (CD11c), the costimulatory molecules CD205 and CD40 from antigen presenting cells, and cells positive for the receptor of natural killer cells in the transplanted (Tx) and the right, non-transplanted lung at day 5 post transplantation.

Acute allograft rejection in orthotopic mouse lung transplants – a scanning electron microscopy study

Draenert A, Jungraithmayr W, Marquart K, Weder W

Objective: Acute rejection (AR) continues to be a major barrier to graft acceptance after lung transplantation (Tx). AR episodes are characterized by mononuclear inflammation targeting small vessels and bronchioles of the transplant. Due to the three - dimensional structure of alveoli and bronchioles, the scanning electron microscopy (SEM) proved to be the method of choice to evaluate these histo-pathological changes. Using an orthotopic single lung transplantation mouse model, this study focuses on the early changes of the transplant compared to the contra-lateral native lung.

Methods: The orthotopic lung Tx mouse model was employed, using C57BL/6 mouse strain as recipient, and Balb/c as donor. At time of harvest (day 1 and 5), explanted lungs were flushed and perfused with glutaraldehyde via the pulmonary artery and the trachea. After preparation under the light microscope, the SEM processes included dehydration, critical point drying and platinum sputtering.

Results: At day 1 post Tx, the allograft showed overall signs of destruction (Fig.A): alveoli were injured with thickening of the alveolar wall containing multiple perforations (single arrow). The capillary lumen between alveoli vanished. There was a widening of the perivascular space around vessels (double arrow) with invasion of inflammatory cells and erythrocytes. Furthermore, bronchioles and alveoli were covered with a thick layer of mucus. In contrast, the non-transplanted lung showed a normal architecture and alveoli and vessels remained in close contact to each other (arrow) (Fig.B/Fig.E).

At day 5 post Tx, the architecture of the alveoli was completely lost with invasion of multiple cells into the alveolar and perivascular space. Leukocytes and macrophages were found numerously in the perivascular tissue, with accumulation of mainly erythrocytes in the alveolar space (Fig. D). The architecture of the non transplanted lung on day 5 was normal. There was found a slightly elevated macrophage invasion in the vascular lumen.

Conclusion: Patho-morphological changes in the lung by an acute rejection could be detected with the SEM –Technique at an early state. The interstitial space, small vessels, alveoli and bronchioles of the allograft were severely altered by invasion of immune cells, with a significant involvement also of the non-transplanted lung.

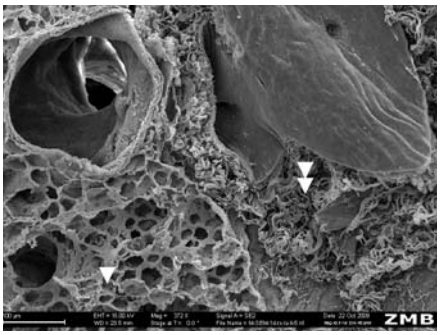


Fig 1: TX d1:1 arrow: destroyed alveoli
2 arrows: Perivascular loosening round the vessel

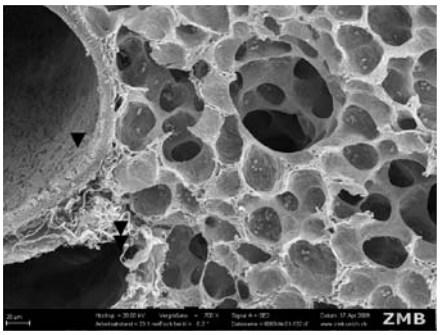


Fig 2: non-TX d1: normal alveolar structure
1 arrow: Bronchiole; 2 arrows: vessel

Fig 3: non-TX d5: vein endoluminal

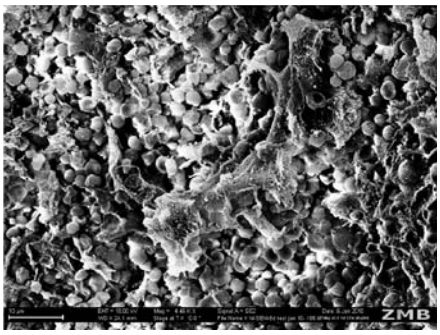


Fig 4: Tx d5: alveolar destruction with cell infiltration

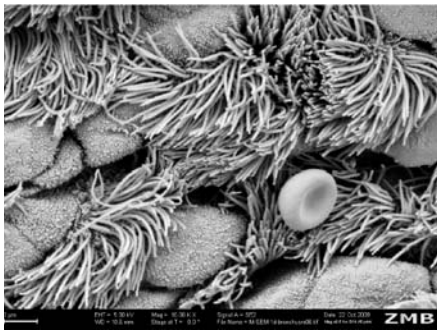


Fig 5: non -TX d1: Normal cilia and epithelium of the native right bronchus

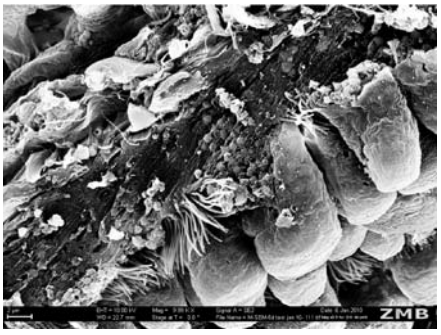


Fig 6: Non-Tx d5: Epithelium of the smaller bronchus with a slight loosening of the basal membrane

Reconditioning of injured donor lungs due to gastric acid aspiration
Inci I, Hillinger S, Erne B, Arni S, Jungraithmayr W, Leskosek B, Weder W

Despite improvements in both donor management and organ preservation, only few of the potential donor lungs are considered acceptable for transplantation. In a pig model of ex-vivo perfusion system we have recently shown that diluted surfactant lavage during ex-vivo perfusion improved the graft function after gastric acid aspiration. Reconditioning of the injured graft due to acid aspiration with diluted surfactant was tested in a pig left lung transplant model.Injured lungs due to gastric acid aspiration are rejected because of the possibility of early graft dysfunction after transplantation. Several experiments have demonstrated that the pulmonary surfactant system is harmed in acute lung injury induced by a variety of conditions.
We hypothesize that diluted surfactant administration will recondition the graft function after acid induced lung injury when given before reperfusion and ventilation in lung transplantation. Lung injury was induced by intratracheal instillation of 1 ml/kg Oroacid® (betaine HCl 400 mg+ Pepsin 100 mg mixture; pH:1.5) by a flexible fiberoptic bronchoscope under direct vision into the left main bronchus during the inspiratory phase of the respiration. In the study groups after induction of lung injury the animals were ventilated 24 hours before harvest followed by 4 hours of cold ischemic storage at 4°C. In control group left lung transplantation was performed without any treatment. In surfactant treatment group the recipients received intratracheal diluted surfactant (1 ml/kg) lavage just before reperfusion and ventilation. Evaluation period was 7 hours. Hemodynamic and ventilatory variables were recorded every hour. Oxygenation index, PaO_2/FiO_2, airway pressure, pulmonary vascular resistance and extravascular lung water index were calculated. In bronchoalveolar lavage (BAL) protein, neutrophil elastase and cytokine levels were measured. The percentage of neutrophils was counted in BAL. Bubble surfactometer was used to measure the surface tension. For histolgical examination the tissue was fixed by instillation of 4% buffered formalin. The specimens will be embedded in paraffin wax, stained with hematoxylin and eosin, and examined under a light microscope.

In these experiments surfactant lavage resulted in lower mean pulmonary artery pressure, lower airway pressure and higher PaO_2/FiO_2 ratio compared to control group (p=0.001) (Figs. 1,2). Although EVLWI was lower in surfactant group the difference was not significant (Fig. 3). Bronchoalveolar lavage neutrophil percentage at the end of the experiment was significantly higher in control compared to surfactant group (p=0.03) (Fig. 4). Minimal surface tension was significantly lower in surfactant group compared to controls (p<0.05) (Fig 5).

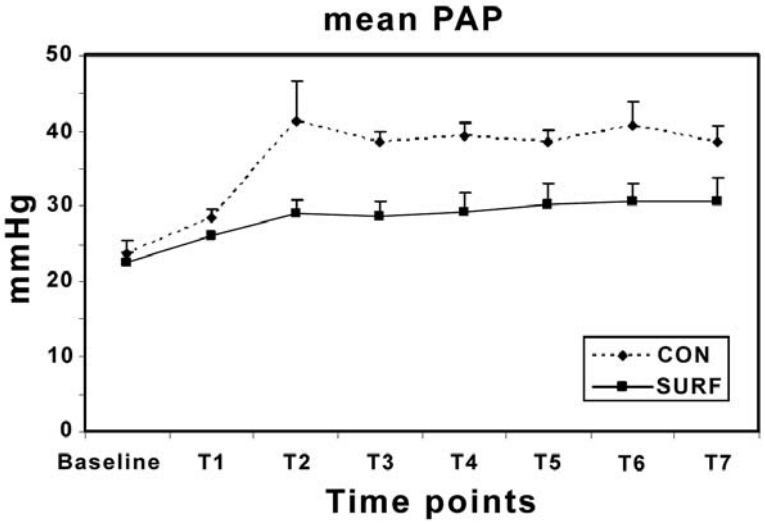


Figure 1. Analysis of variance (ANOVA) for repeated measures which consists of all measurements made during the reperfusion period for mean pulmonary artery pressure significantly differed between the groups (p=0.01). mPAP: Mean pulmonary artery pressure Time points: Baseline, BO: 10 min. before occlusion of the right lung, AO: 10 min. after occlusion of the right side, T1: 1 h perfusion with two lungs, T2: 1 h after occlusion of the right side, T3: 2 h after occlusion of the right side, T4: 3 h after occlusion of the right side, T5: 4 h after occlusion of the right side, T6: 5 h after occlusion of the right side, T7: 6 h after occlusion of the right side.

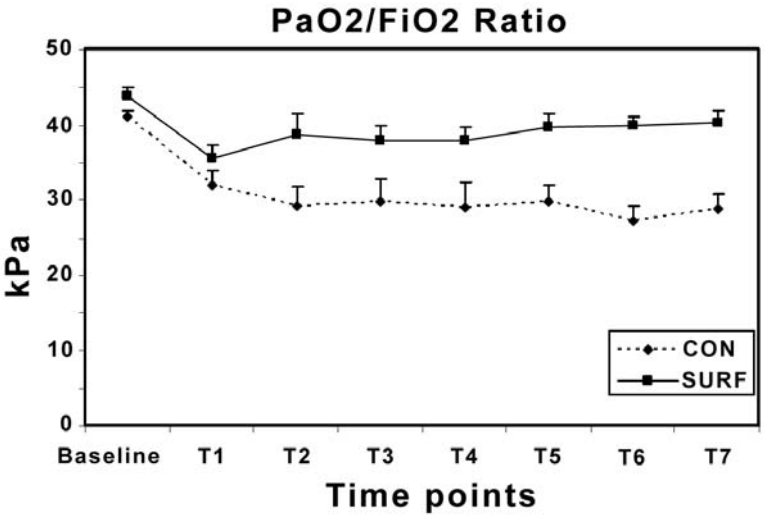


Figure 2. Analysis of variance (ANOVA) for repeated measures which consists of all measurements made during the reperfusion period for oxygenation significantly differed between the groups (p=0.001). Oxygenation: Partial arterial oxygen pressure/Fraction of inspired oxygen (PaO2/FiO2 ratio). Time points as in Fig. 1.

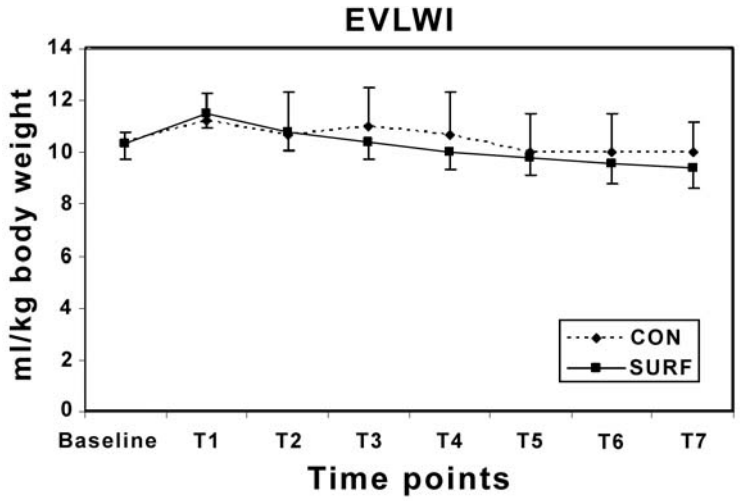


Figure 3. Extravascular lung water index (EVLWI) was higher at all time points during the reperfusion for the CON group. Time points as in Fig. 1.

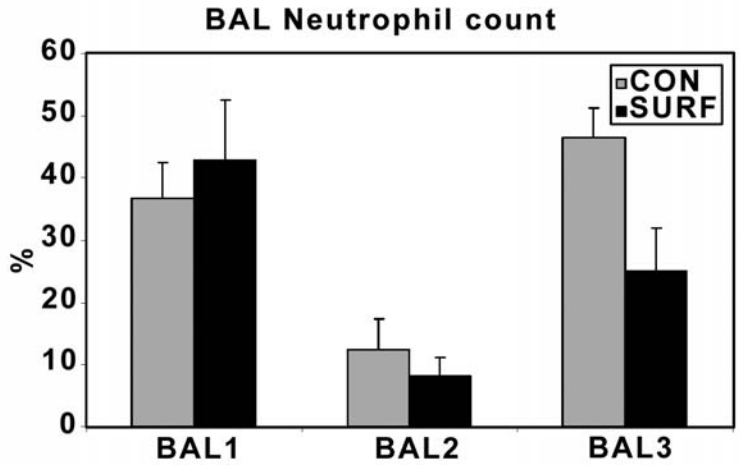


Figure 4. BAL Neutrophil count at the end of experiment was significantly higher in the control compared to Surfactant treated group (p=0.03). BAL 1: 24 hours after acid injury (left lung), BAL 2: right lung without injury. BAL3: Transplanted left lung, at the end of experiment.

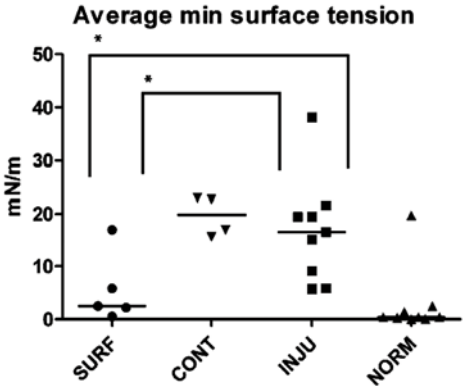


Figure 5. Minimal surface tension. INJU: Donor left lung 24 hours after ventilation following acid injury. NORM: Right lung of the same animal 24 hours after ventilation.

In conclusion, this experiment showed that surfactant instillation following acid aspiration protected the lung graft resulting in better graft function.

Effect of N-Acetylcysteine on acute allograft rejection after lung transplantation

Erne B, Jungraithmayr W, Arni S, Weder W, Inci I

Background. Although the outcome of transplantation have improved impressively during the last few years, primary graft dysfunction and rejection still remain primary causes of increased morbidity and mortality after human lung transplantation. Recently, we demonstrated a significant reduction of ischemia/reperfusion injury using N-Acetylcysteine, an antioxidant, after lung transplantion in animal models. The present study was designed to find out an additional protective effect of N-Acetylcysteine on acute lung rejection.

Methods. Orthotopic left lung transplantation was performed from Brown Norway to Lewis rats.. Recipients were randomized in 2 groups: N-Acetylcysteine treated (NAC) or control (CON). In each group observation was done for 1 and 5 days. In treated groups (NAC1 and NAC5), N-Acetylcysteine (NAC) was given 150 mg/kg intraperitoneally 30 minutes before explantation and reperfusion of the graft. The intraperitoneal injection of NAC was repeated daily until euthanasia. Transplanted and native lungs were assessed for histology, immunohistochemistry, reduced glutathione and activated NF-kappa B levels.

Results. Significant higher tissue level of glutathione was found in NAC group on day 5. High activation level of NF-kappaB was measured in CON5 grafts (0.66 ± 0.07 OD), which is significantly different from those of NAC5 grafts (0.49 ± 0.05 OD; $p = 0.03$). Immunohistochemistry revealed a significant higher appearance of CD68 positive macrophages in CON5 compared to NAC5.

Conclusion. Donor and recipient treatment with N-Acetylcysteine has a significant influence on acute rejection after transplantation, most likely mediated through the NF-kappaB pathway.

Achievements 2009

- Prize of the swiss society of thoracic surgery for best publication 2009 to Wolfgang Jungraithmayr
- Teaching of the „Mouse Model of Orthotopic Lung Transplantation“ at Layola University, Chicago, USA; John’s Hopkins University, Baltimore, USA; Leuven-University, Leuven, Belgium.
- Swiss National Fond Grant with the title: "The Role of CD26/DPP IV and SDF-1 in pulmonary ischemic injury in a mouse lung transplantation model" to Wolfgang Jungraithmayr

Collaborations:

- Antwerp, Belgium: CD26/DPP IV
- Dept. of Thoracic Surgery, Layola University, Chicago, USA

Selected references:

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2.6.2 Oncology


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

Wiedl T, Arni S, Hillinger S, Weder W

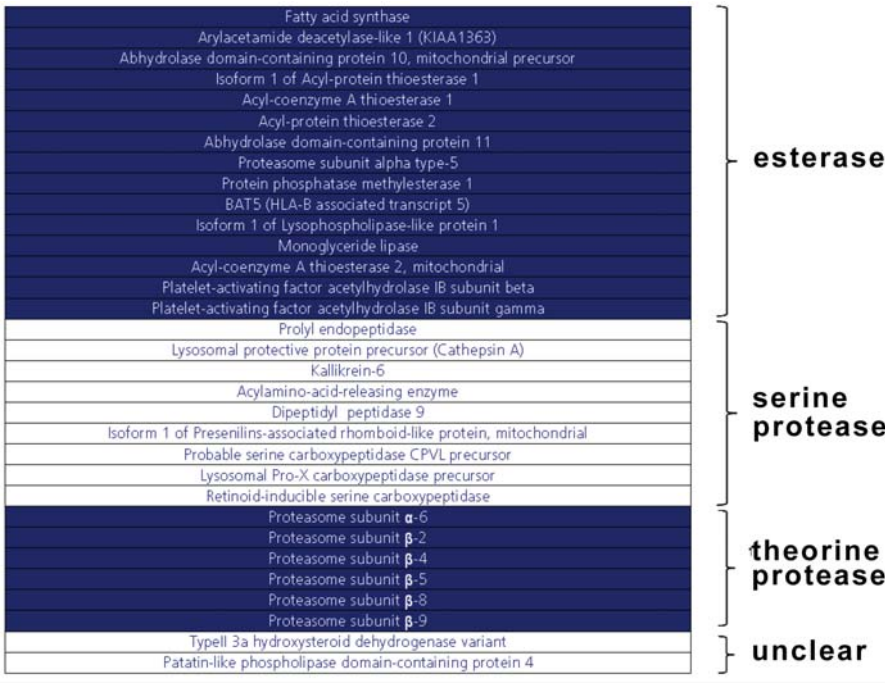
Lung cancer is the major cause of all cancer-related deaths with overall survival rates not having improved significantly over the past 20 years. Therefore establishing predictive molecular markers that may also represent potential therapeutic targets at the same time is of utmost importance. In the post genomic era mRNA expression profiling and proteomics are employed to discover biomarkers. One drawback of these techniques is that both strategies aim to measure mere abundances instead of real enzymatic activities. A new methodology termed **Activity Based Protein Profiling (ABPP)** circumvents these limitations by employing active site directed chemical structures, so-called Activity Based Probes (ABPs) to selectively target active enzymes – but not their inactive versions – thereby making a direct read-out of activity profiles of any given proteome possible. This study aims to investigate activity profiles of serine hydrolases (SHs) in human lung adenocarcinoma biopsies. The SH superfamily represents a large and highly diverse enzyme class, members of which have previously been reported to play a role in lung cancer development. **This study aims to establish ABPP in a clinical environment and to investigate the potential of SH activity profiles as predictive biomarkers in lung adenocarcinoma.**

Methods A directed mass spectrometry (MS) based approach was used for qualitative and quantitative analysis of ABP tagged proteomes. After incubation of proteomes with FP-Biotin (the Activity Based Probe used to target serine hydrolases (SHs)) and enrichment using avidin coated agarose beads, SHs were incubated with trypsin and tryptic peptides were analyzed on an FTICR mass spectrometer (LTQ-FTMS, Thermo Finnigan, Bremen, Germany). MS/MS data were searched against a recently updated human database (UniProt) using the Mascot 2.2 search engine. The parameters for precursor tolerance and fragment ion tolerance were set to 5 ppm and 0.8 Da, respectively. For quantitation MS data were analyzed with SuperHirn v0.3. Only peptides with a PeptideProphet probability > 0.9 were included in the analysis. After initial experiments in data-dependent acquisition (DDA) mode, inclusion/exclusion lists were generated and MS/MS spectra were recorded accordingly to these lists since then. Only identifications that were absent in control reactions (ABP left out) were considered positive hits

Results and Discussion

Table 1 shows a representative panel of serine hydrolases that have been identified with ABPP in the human lung adenocarcinoma cell line CaLu-3. Approximately 30 SHs – mostly esterases and proteases – are identified per investigated proteome, regardless whether proteomes are derived from cell lines or human lung adenocarcinoma biopsies. Certain enzymes like Fatty Acid Synthase (FAS) or Kallikrein-6 (KLK6) have previously been associated with lung cancer development or lung cancer diagnosis, respectively.

However, further enzymes like Arylacetamide deacetylase like-1 (AADACL1) have not been linked to lung cancer so far. Interestingly, several threonine proteases constituting the proteasome were identified, indicating that this this class of enzymes is also susceptible to the ABP FP-Biotin. Activity Based Protein Profiling (ABPP) has been established as a biomarker screening platform in a clinical environment. **The methodology allows a relative fast and semi-quantitative analysis of enzymatic activity profiles in cell lines and primary human specimen.** Within this study activity profiles of serine hydrolases are investigated, nonetheless the established technique is applicable to any enzyme class for which ABPs have been developed. The implemented methodology will now be employed to screen >60 fresh-frozen human lung adenocarcinoma biopsies and corresponding normal tissues for SH activities. In combination with clinical follow-up data, the results of this study will ideally allow the discrimination of low-/ high-risk lung adenocarcinoma patients with respect to treatment response and survival.



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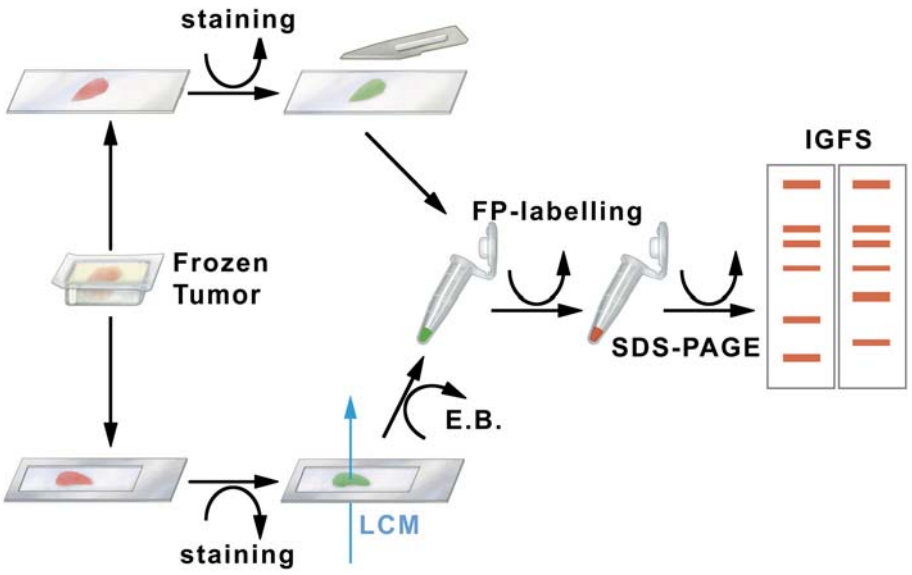
Laser-Capture Microdissection Impairs Activity-Based Protein Profiles for Serine Hydrolase in Human Lung Adenocarcinoma
Collaud S, Wiedl T, Arni S, Hillinger S, Weder W

Laser-capture microdissection (LCM) enables the selection of a specific and pure cell population from a heterogenous tissue such as tumors. Activity-based protein profiling (ABPP) is a chemical technology using enzyme-specific active site-directed probes to read out the functional state of many enzymes directly in any proteome. The aim of this work was to assess the compatibility of LCM with downstream ABPP for serine hydrolase (SH) in human lung adenocarcinoma. Fresh frozen lung adenocarcinoma tissue was stained with hematoxylin, toluidine blue, or methyl green (MG). Proteome from stained tissue was labeled further with SH-directed probes, and ABPPs were determined on a one-dimensional gel-based approach. This allowed us to assess the impact of staining procedures on their ABPPs. The effect of the LCM process on ABPPs was assessed furthermore using MG-stained lung adenocarcinoma tissue. The staining procedures led to strong changes in ABPPs. However, MG staining seemed the most compatible with downstream ABPP. MG-stained, laser-captured, microdissected tissue showed additional change in profiles as a result of the denaturing property of extraction buffer but not to the microdissection process itself. LCM staining procedures but not microdissection per se interfered with downstream ABPP and led to a strong change in ABPPs of SHs in human lung adenocarcinoma.



General experimental workflow from the fresh frozen biopsy to the detection of ABPPs. E.B., Extraction buffer; FP, fluorophosphonate probe; IGFS, in-gel fluorescence scanning.

Immunotherapy for lung cancer
Hillinger S, Arni S

We previously reported an efficient treatment based on the injection of both the commercially available chemokine CCL19 and cytokine IL7 to eradicate lung tumors in murine models. We will produce murine myeloid dendritic cells (mDC) expressing both the chemokine CCL19 and IL7 via adenoviruses. Our final goal is to inject therapeutically transformed mDC in tumour bearing mice. In the 2008 annual report we presented the preparation of an adenovirus pseudotyped with an RGD modification at the surface and expressing mCCL19. It appears that despite the high titer of adenoviruses obtained, the expression of the chemokine CCL19 was lost during amplification and mCCL19 undetectable by ELISA. We decided to redesign a non pseudotyped adenoviruses by using the AdEasy system developed in the laboratory of Bert Vogelstein[1].
During this year we produced five adenoviruses designed Adeasy-CMV-mCCL19 and Adeasy-CMV-mIL7. In parallel we constructed green fluorescence protein variant designed Adeasy-CMV-mCCL19_IRES_GFP and Adeasy-CMV-IL7_IRES_GFP and a control Adeasy-CMV_IRES_GFP. This modification allow us to monitor infection both during the amplification and production steps. We obtained high titer of recombinant adenoviruses following a four steps amplification protocol described by the laboratory of Bert Vogelstein[2]. The fourth amplification with a high titel of recombinant adenoviruses were obtained after 3 months. The viral titer calculated from the endpoint dilution were 1E+08 to 1E+08 pfu/ml (Table 1). The expression of mCCL19 and mIL7 were determined with specific ELISA kits (R&D system Duoset sandwich elisa) following the manufacturer's instructions. As reported in Table 1, high concentration of mCCL19 and mIL7 were obtained after infection of A549 cells.

Adenovirus clone and amplification stage	Adenovirus titel [pfu/ml]	mCCL19 [pg/ml]	mIL7 [pg/ml]
Clone 257 fourth amplification Adeasy-CMV-mCCL19-IRES-GFP	1.995E+08	923.8	0
Clone 86 fourth amplification Adeasy-CMV-mIL7-IRES-GFP	1.259E+09	0	3063.8
Clone 22 fourth amplification Adeasy-CMV-IRES-GFP	1.00E+09	0	0
Clone 46 second amplification Adeasy-CMV-mCCL19	1.995E+07	944	0
Clone 18 second amplification Adeasy-CMV-mIL7	3.162E+07	0	1834

Table 1:
Significant titel and concentration of mCCL19 and mIL7 proteins were obtained without cross contamination.

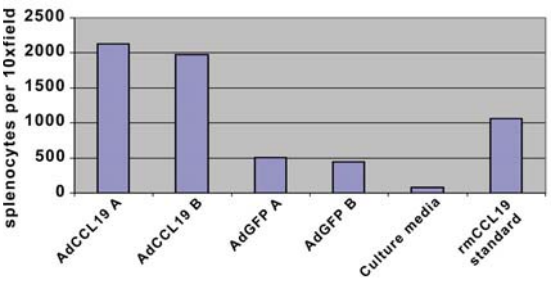


Figure 1: Chemotaxis of mouse stimulated splenocytes is induced by different preparations of AdCCL19 and rmCCL19.

High concentration of mCCL19 obtained in adenovirus infected culture supernatant were also tested in a bioassay for the chemotaxis of mCCL19 on stimulated splenocytes through a Boyton chamber (Figure 1).

We next tested adenoviruses infection of mDC. Since mDC are known to be poorly infected by adenoviruses we performed protocol based on 120 minutes centrifugation[3] at 2000 g or on calcium precipitation[4]. Our results with centrifugation of cells in high titel of adenoviruses show that less than 1% of the primary mDCs were infected (Figure 2 A to F). Similar infection rate were observed following calcium precipitation (not shown).

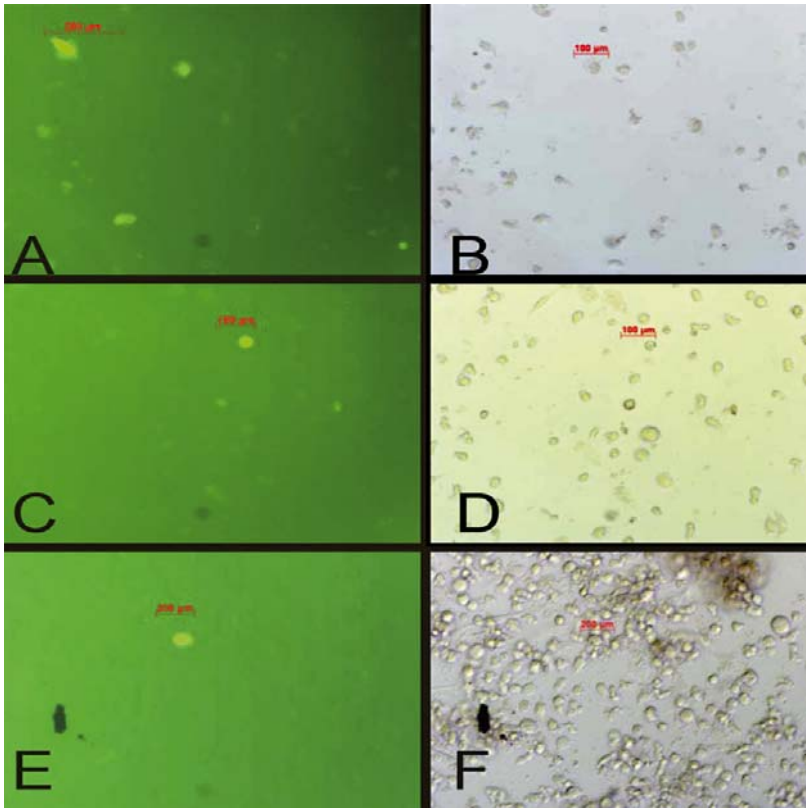


Figure 2: C57BL/6 mDCs infected with AdCCL19_IRES_GFP (A and B) or AdIL7_IRES_GFP (C and D). BALB/c mDCs infected with Ad_GFP (E and F). The multiplicity of infection shown are 100. Images are at 20x magnification and shown 36h post infection.

We demonstrate that our five AdEasy based adenoviruses generate functional levels of mCCL19 and mIL7. These findings strengthen the rationale for further investigation in DC transfection by adenoviruses coding for mCCL19 and mIL-7 as a DC-based therapy in cancer treatment.

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Achievements 2009

- 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
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- 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

Selected references:

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Malignant pleural mesothelioma –intrapleural therapy after surgery
Opitz I, Erne B

Background: 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. As reported previously a small animal recurrence model was established in a rat and cisplatin was evaluated as intrapleural therapy. Cisplatin-solution and cisplatin bound to an autologous fibrin-carrier (Vivostat) effectively reduced local tumour recurrence after tumour resection. In the underlying project both compounds were compared as to their pharmacodynamic characteristics in a large scale pig model.
Materials and Methods: A randomized comparison of intrapleural chemotherapy with cisplatin-fibrin versus intrapleural cisplatin solution after left-sided pneumonectomy plus parietal pleurectomy was performed in a pig model. One group received 90 mg/m^2 intrapleurally (control) as a solution mixed with NaCl 0.9% and the other group received 5mg cisplatin combined to the fibrin sealant Vivostat® applied on a predefined area of 10x10 cm. Blood samples will be taken before treatment (baseline), at the end of instillation of cisplatin, and at 1, 2, 4, 8, 12, 24 hours and after 2 and 5 days following intrapleural treatment. For the analysis of platinum concentration in the blood, haemoglobin, haematocrit, white blood cells, platelets, urea, and creatinine, liver parameters (LDH, GOT, GPT, yGT, AP). Superficial and deep pleural biopsies will be assessed for platinum concentration in the pleural tissue after 2 and 4 hours and by VATS pleural biopsy at day 2 and 5 after application of cisplatin intrapleurally. Urine samples will be collected before treatment and from 2- and 4-hour pools, and the volume of urine for each collection period will be recorded. Pathological analysis of the pleural tissue and the kidneyas will be performed at the end of the experiments. The samples of the pleural tissue were homogenized and analyzed for determination of the drug level and the concentration-time curve (AUC). The level of total platinum will be measured by means of inductively coupled plasma sector field mass spectrometric detection with a matrix-matched calibration procedure in the Department of Anorganic Chemistry, ETH. For comparison of the AUC of both groups dose-correction will be performed.
Results: The dose- and surface-corrected mean concentration of cisplatin in the chest wall tissue 2 h after the application was 504.1 mg/L in animals treated with cisplatin-fibrin (geometric coefficients of variation, CV, 88%), compared to 249.1 mg/L (CV 261%) in the control group. Five days after the application, mean concentrations in the tissue were 72.5 mg/L (CV 216%) and 21.8 mg/L (CV 427%) in fibrin- and solution treated animals, respectively. In plasma, the dose- and application surface-corrected exposure towards cisplatin (area under the concentration-time curve from 0 – 5 d after surgery)

was clearly and significantly lower with cisplatin-fibrin than with cisplatin-solution: 68.5 mg/L*h (CV 28%) versus 755.8 mg/L*h (CV 110%). This is also reflected by significantly reduced serum-creatinine values in the study group in comparison to the control group as well as significantly better well-being scores for the animals treated with cisplatin-fibrin at each day of the observation (all $p<0.05$).

Conclusions: : Cisplatin tissue concentration after cisplatin-fibrin treatment was at least two fold higher at 2 h and 5 d while systemic cisplatin concentrations were significantly reduced. This finding offers a clear advantage since rate and severity of systemic adverse events can be reduced while local cytotoxic concentrations are at least maintained, what will be soon evaluated in a phase-I study

Outlook: Further experiments with application of cisplatin-fibrin to the whole pleural surface and the mediastinum will be performed to assess toxicity and efficacy. In the next step a clinical phase-I study is planned.

Achievements 2009

- Krebsliga grant, ESMO Award

Collaborations:

- Department of Oncology (Emmanuela Felley-Bosco, Rolf Stahel)
- Institute for Biostatistics (Burkhardt Seifert)
- Department of Anorganic Chemistry, ETH (Christopher Latcozy, Detlef Günther)

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

Frei C, Opitz I, Felley-Bosco E

Background: 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. Since we found that stem cell signaling is present in vivo and is maintained in primary culture, the aim of our study is to identify cancer stem cells which could specifically be targeted for treatment.

Methods and Results: 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 diff-erentiation markers mesothelin and N-cadherin. Both SP and NSP were tumorigenic, however the SP derived tumors had 90% epithelioid and 10% sarcomatoid phenotype compared to NSP-derived tumors which were 100% epithelioid.

Conclusion: Taken together these results indicate that SP and NSP from ZL55 cells contain different populations of tumorigenic cells and we are currently testing whether SP and NSP-derived tumors can both give rise to tumors themselves.

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

Induction of tumoral senescence by neo-adjuvant chemotherapy of malignant pleural mesothelioma and association with clinical outcome: an exploratory analysis

Sidi R, Pasello G, Opitz I, Felley-Bosco E

Purpose: The aim of this study was to assess the expression of senescence versus apoptosispathways in malignant pleural mesothelioma (MPM) tumor samples before and after neoadjuvant platinum-based chemotherapy and to investigate their relationship with clinical outcome.

Experimental Design. Specific senescence pathways were assessed by quantifying the expression of p21 and 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. p21 and PAI-1 expression was also assessed by immunohistochemistry. In addition, beta-galactosidase activity staining at pH 6.0 was performed. Apoptosis was determined by TUNEL assay. Clinical outcome was assessed by modified RECIST criteria and progression-free and overall survival.

Results: In a training set (n=9 patients) paired comparison demonstrated a significant increase in senescence score ($p<0.05$ for the p21-PAI-1 and $p<0.02$ for the general senescence score) and apoptosis ($p<0.01$) after chemotherapy. The patients with the highest increase in senescence score had stable disease, while patients with little change in senescence score accompanied by a high increase in apoptosis had an objective response after chemotherapy. The hypothesis that stable disease might be associated with an increased senescence was confirmed in a tissue microarray (n=26 patients) using p21-PAI-1 immunohistochemistry as readout. In the 21 patients where survival data was available, increased senescent was significantly associated with a worst outcome.

Conclusions: Our results demonstrate induction of senescence by neo-adjuvant chemotherapy in a proportion of patients with MPM and its potential association with a poor outcome.

Collaborations:

- Department of Oncology (Emanuela Felley-Bosco, Claudia Frey, Roy Sidi, Giulia Pasello, Rolf Stahel)
- Department of Clinical Pathology (Alex Soltermann, Holger Moch, Peter Vogt)
- Institute for Biostatistics (Burkart Seifert)

Prognostic Marker for Malignant Pleural Mesothelioma
Opitz I, Schramm A

Background: 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.

Patients and methods: 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.
Results: 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), therapy (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.
Conclusion: 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.

Outlook: In a collaboration project with Dublin the expression of other marker (RON) will be analysed for the prognostic significance for MPM patients in this historical data set.

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

Opitz I, Schramm A

Background: 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.

Patients and Methods: 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.

Results: 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 ($p=0.7$).

Conclusion: 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.

Outlook: PTEN and other marker will be assessed in our prospective database of patients that underwent induction chemotherapy followed by extrapleural pneumonectomy and pre- and post-chemotherapy biopsies will be compared. Furthermore FISH and mutation analysis will be performed.

The value of ERCC1 as predictive and prognostic marker for Malignant Pleural Mesothelioma

Opitz I, Schramm A, Tutic M

Background: 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.

Patients and Methods: 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 semiquantitatively 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 2010, 167 were intended to be treated with induction chemotherapy (40% cisplatin/gemcitabine; 60% cisplatin/pemetrexed) followed by extrapleural pneumonectomy (EPP). Response to chemotherapy according to modified RECIST criteria was available for 89 patients. One TMA with tumour of 126 MPM patients who underwent induction chemotherapy followed by EPP was constructed (post-CTX). Another TMA with 110 patients where pre-chemotherapy biopsies (pre-CTX) were available was constructed. ERCC1 expression was assessed and correlated to prospectively documented data. The influence on overall survival (OAS) and response to chemotherapy was evaluated.

Results: 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-score ³ 260 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.

ERCC1 was expressed in > 90% of the pre-and post CTX biopsies. The expression score changed from a median score of 2.8 in the pre-CTX- to a median score of 2 in the post-CTX biopsies. There was no correlation between ERCC1 expression and the response to chemotherapy assessed by modified RECIST criteria. The median overall survival of all 167 patients was 19 months, of the 116 patients undergoing EPP 22 months. Neither pre- nor post CTX ERCC1 - and also not the ERCC1 change of expression global score showed significant influence on OAS.

Conclusion: Loss of ERCC1 expression was shown to be an independent prognostic marker for poor overall survival of mesothelioma patients without standardized treatment. The prognostic role of ERCC1 expression for OAS was not confirmed in mesothelioma patients treated with induction chemotherapy followed by EPP. A predictive role for response to chemotherapy was not proven.

Outlook: Pre-chemotherapy pleural biopsies will be analysed for ERCC+expression and the change after induction chemotherapy will be assessed in pleuropneumonectomy-specimens.

Volumetry – an alternative tool for assessment of therapy response after induction chemotherapy for malignant pleural mesothelioma

Opitz I, Tutic M

Background: To assess tumor volume per se and its response to chemotherapy, adequate methods are necessary. Nowadays there is no satisfying “gold standard” technique for tumor measurement in MPM. The reason for this is the “rind-like” and often irregular growth of the pleural mesothelioma providing major challenges for adequate tumor assessment. The purpose of this study was to assess the value of volumetric measurement of malignant pleural mesothelioma (MPM) in our multimodality therapy setting compared to modified RECIST, which are the current standard for assessment of therapy response.
Method: Thirty patients with proven MPM were included. All patients underwent chest CT scan before and after 3 cycles of induction chemotherapy to evaluate therapy response. Three readers assessed independently tumor response using two different methods: Modified RECIST criteria and tumor volumetric approach. Tumor volumetry was performed using dedicated software.
Inter-rater reliability of uni-dimensional and volumetric measurements was assessed using analysis of variance. Uni-dimensional and volumetric measurements were correlated. Tumor response classification for modified RECIST was compared to volumetric approach applying uni-dimensional RECIST volumetric equivalent criteria.
Results: The determination of uni-dimensional tumor measurement revealed a low inter-rater reliability (0.55) A high inter-rater reliability (0.99) was found for absolute tumor volumes.
The number of cases classified as “stable disease” was higher for volumetry approach using tumor equivalent criteria compared to modified RECIST.

Conclusion: Volumetric measurement of MPM is a robust and sensitive method to measure therapy response. Further prospective studies are needed to define volumetric specific tumor response criteria.

Achievements 2009

- Krebsliga grant, Budget Schmidheiny, Matching Fund

Collaborations:

- Department of Oncology (Emmanuela Felley-Bosco, Rolf Stahel)
- Department of Clinical Pathology (Alex Soltermann, Holger Moch, Peter Vogt)
- Institute for Biostatistics (Burkhard Seifert)
- Institute of Radiology (Thomas Frauenfelder)

Selected references:

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- Malignant pleural mesothelioma. Stahel RA, Felley-Bosco E, Opitz I, Weder W. Future Oncol. 2009 Apr; 5(3): 391-402. Review

2.7 Urological Research

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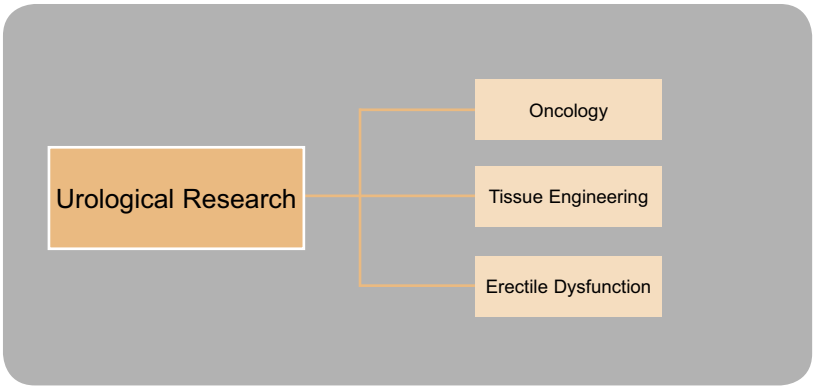
Scientific Coordinator



2.7.1 Oncology

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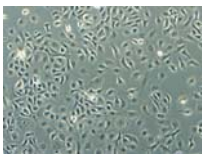
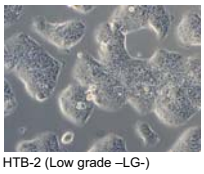
The prognostic value of correlations between lymph-angiogenesis, lymph-node metastasis and tumour staging in bladder cancer
Poyet C, Thomas L, Provenzano M

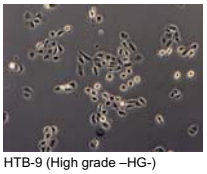
Lymph angiogenesis has been seen to play a role in the promotion of tumour spreading, particularly bladder cancer. There is thus a high interest in identifying markers that can diagnose bladder cancer with high risk of progression. Vascular endothelial growth factors (VEGFs) have been suggested to determine a state of cancer invasion and dissemination by modulating lymphatic vascular markers with a central role in lymphatic vessel development and maturation. In addition, chemokine-receptor CCR-7 and two secondary lymphoid chemokines (CCL19 and CCL21) secreted by lymphatic endothelial cells (LECs) in relation to VEGF-C/VEGFR3 axis, have also been relevantly indicated in tumour lymph-angiogenesis. Our preliminary data set the stage for a possible relevant difference between high-grade and low-grade bladder cancer for lymph-angiogeneic markers gene profiling. We have tested VEGF-A, -C, -D and CCR-7 gene expression (qRT-PCR) on three different bladder cancer cell lines (HTB-2, -4, and -9), as compared to normal urothelium (Uro-Tsa; Figure 1) finding that VEGF-C is higher expressed in high-grade bladder cancer cell lines HTB-4 or T-24 (p=0.0003) and HTB-9 (p=0.005), while VEFG-A is higher expressed in low-grade cancer cells HTB-2 (p=0.001; Figure 2).



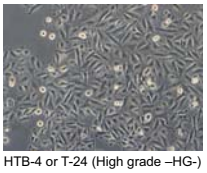
Uro-Tsa (Normal Urothelial)



HTB-2 (Low grade –LG-)



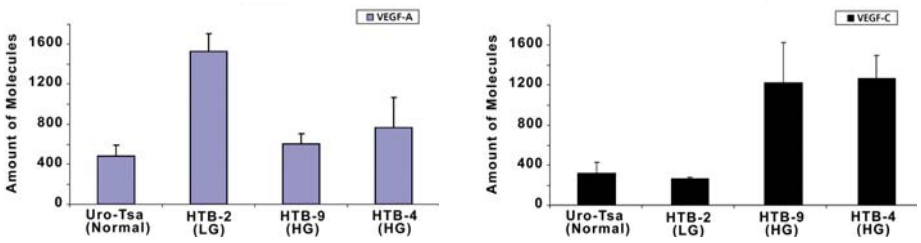
HTB-9 (High grade –HG-)



HTB-4 or T-24 (High grade –HG-)

Figure 1: Different bladder cell lines used in the study.

Figure 2: VEGF-A and -C gene expression in all four bladder cell lines tested



Relevant is the over-expression of CCR-7 in low-grade bladder cancer cells ($p<0.0001$; Figure 3), data correlating with the possible tendency (10-20% of cancers) of low grade bladder cancer (NMIBC) to upgrade into the higher grade stage (MIBC). The finding is fortified by the reduced expression of Uroplakin II (marker for normal urothelium and bladder carcinogenesis) in high-grade cancer cell lines ($p=0.0002$) and by the relevant higher expression of serine protease maspin (marker for metastatization) in low-grade cell lines ($p=0.007$; Figure 4).

Figure 3: Fold increase of VEGFs and CCR-7 genes in bladder cancer cells

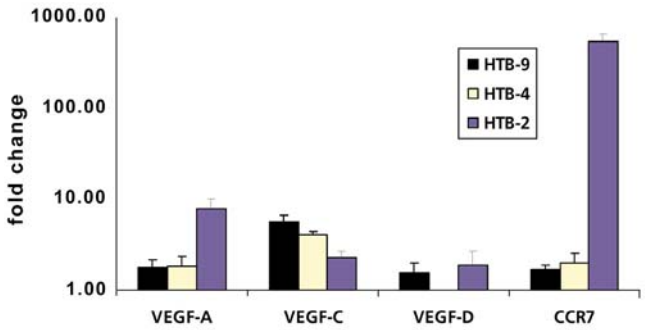
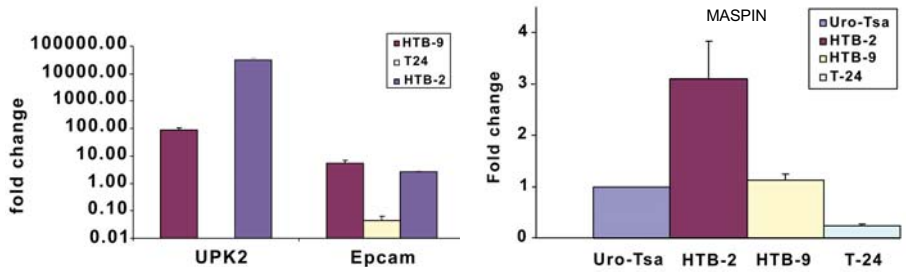


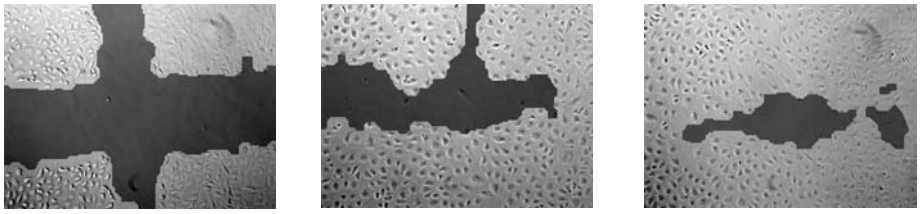
Figure 4: Uroplakin II, EpCam and Maspin gene expression in bladder cancer cells



In addition, we also started to investigate on genes involved in the development, maturation and cell-cell interaction of LECs. We cultured LECs in type I collagen coated culture dishes with endothelial cell basal medium. The modulation of three relevant LECs genes upon VEGF-C stimulation (100 ng/ml for 24 hours) was tested: 1) lymphatic vessel endothelial hyaluronan receptor-1, LYVE-1: the most specific lymphatic endothelial markers for lymphatic development; 2) Angiopoietin 2, Ang-2: thought

to have a role in the maturation step of lymphatic vasculature; 3) chemokine CCL21: secreted by LECs has been shown to promote migration of CCR7-expressing metastatic malignant melanoma cells. We observed 20-fold up-regulation of Lyve-1, 2.3-fold up-regulation of Ang-2 and 2.5-fold up-regulation of CCL21, compared to the negative control (previous test on LECs gene modulation by culturing cells over time -3 to 48h- without stimulation: no relevant changes). For migration assay, LECs cultures were scratched after cell attachment with a 200µL sterile pipette tip and extensively washed with PBS to remove detached cells and debris. Two crosses were scratched in each well and instantly center-imaged at 5× magnification. Cells were then incubated in serum-reduced or full-growth medium (VEGF-C). After 24h, medium was totally replaced with PBS and images of same areas were acquired. Figure 5 shows representative pictures created during migration assay with or without VEGF-C stimulation. Data were evaluated using the Tscratch Software with default parameter settings.

Figure 5
A) LECs after scratching (time 0) B) LECs after 24h (no stimulation) C) LECs after 24h (VEGF-C)



Differences in percentage of closure rate upon stimulation are shown. Higher migration potential of LECs upon either VEGF-A and VEGF-C stimulation are reported. VEGF-D activity is currently under evaluation. Our preliminary data suggest that LECs differentiation, maturation or migration could be prevalently managed by VEGFs involvement, in term of time of exposure and concentration. Furthermore, the up-regulation of CCL21 upon VEGF-C confirms its possible role in tumour cell migration via CCR-7 triggering on tumour cells. Both the higher migration potential of LECs upon VEGF-C induction and VEGF-C-driven up-regulation of genes responsible for LECs activity point out on the fundamental role of VEGF-C, possibly in bladder cancer. A better understanding of lymphatic tumour dissemination and tumour-induced lymphangiogenesis in bladder cancer might lead to new therapeutic applications. The expression of these factors has not been comparatively evaluated in all different bladder tumour grades and stages and their potential prognostic significance has not been indeed fully explored.

Achievements 2009

- The 1st Clinical Research Retreat, Maienfeld, Switzerland, NOV 19-21, 2009

Collaborations:

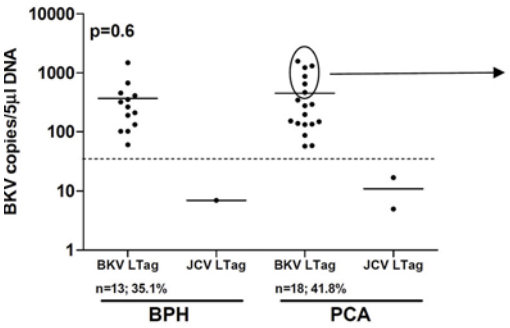
- Molecular Tumour Pathology, Department for Surgical Pathology, University Hospital of Zürich.
- Institute of Pharmaceutical Sciences, ETH, Zürich.

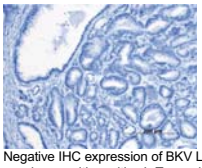
Polyomavirus BK L-Tag specific immune regulatory activity correlates with L-Tag positive cancer lesions and biochemical recurrence in prostate cancer patients

Sais G, Anzivino E, Provenzano M

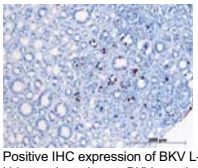
The polyomavirus BK (BKV) large tumor antigen (L-Tag) contributes to oncogenesis by regulating crucial pathways of human cell cycle, such as p53 activity, when non permissive cells are infected. Therefore, L-Tag has been identified as an important target of immune surveillance in L-Tag expressing tumors. In prostate, BKV has been detected at pre-early cancer stages thus inducing us testing whether an immune response against L-Tag-p53 binding regions might define a role for BKV immune surveillance in prostate cancer (PCa). Either consecutive newly diagnosed PCa patients (n=43) or age-matched benign prostatic hyperplasia patients (n=37) were enrolled. Gender-matched healthy donors were used as control. Clinical data were collected by retrospective review of patients' files. Five year follow-up after surgery with early censoring was performed on 32 (74%) of 43 PCa patients defining biochemical recurrence (BR+) as the first PSA 0.2 ng/ml or greater (biochemical freedom from failure at 5 years was 84.4%). Detection of BKV L-Tag DNA was carried out in surgically excised PCa specimens and BKV negative lesions were defined upon analysis of three random punches in the tumor area (positive control: transplanted kidney with allograft BKV nephropathy.

Figure 1: BKV L-Tag molecular testing in tissue specimens from either PCa or BPH patients, as compared to BKV L-Tag expression by IHC





Negative IHC expression of BKV L-Tag in one of PCa tissue specimens with positive L-Tag molecular testing



Positive IHC expression of BKV L-tag in the control transplanted kidney rejected upon BKV reactivation

Systemic immune regulatory profiling was tested upon BKV L-Tag peptide-pool induction and analyzed using both cytokine gene expression and regulatory T cell (Treg) expansion (n=22).

Figure 2: Cytokine gene profiling upon L-Tag pool induction

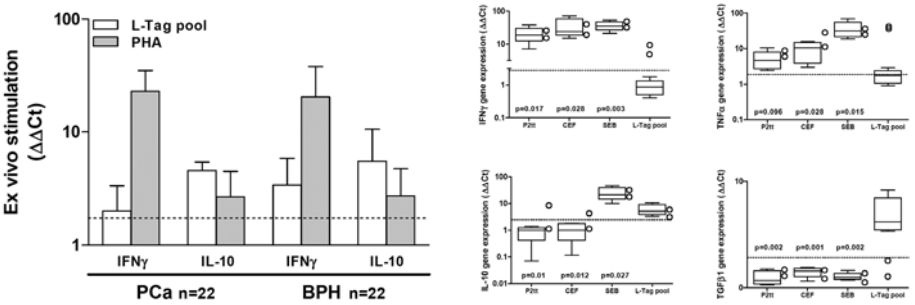
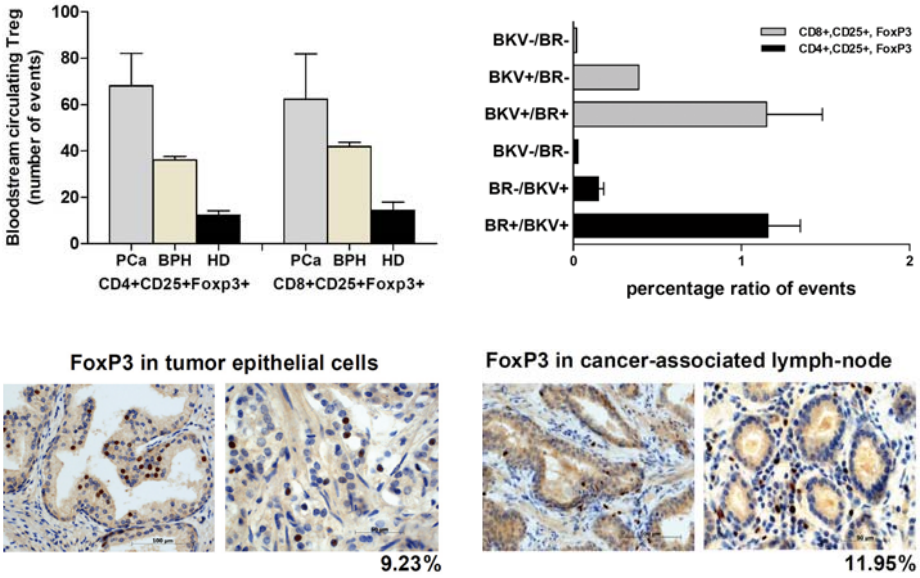


Figure 3: Naturally occurring or L-Tag antigen-specific regulatory T cells in PCa patients



The two cohorts of PCa patients (BR+, n=5 and BR-, n=17) were related to patients' clinical characteristics (PSA, Gleason score; GS), BK viral features and patients' immunology. We found a strong correlation between BR status and both L-Tag expression in tumor specimens and L-Tag serology ($p<0.002$; Table 1). Cytokine gene profiling upon L-Tag induction was positively correlating as well ($p<0.005$). Table 2 shows all BR+ PCa patients with positive L-Tag molecular testing, positive L-Tag serology and a relevant immune regulatory activity. This immune regulatory trend could be confirmed by the evidence of L-Tag peptide specific Treg cells in BR+ PCa patients.

Table 1:

Pattern	*χ2(Pearson)*	*alpha*	*pts*
BR vs Gleason score	*0.02*	*>0.5*	*n=18*
BR vs PSA (ng/ml)	*1.03*	*>0.5*	*n=22*
BR vs L-Tag molecular testing	*5.96*	*<0.02*	*n=22*
BR vs immune regulatory profiling	*4.95*	*<0.05*	*n=17*
BR vs LTag serology (IgG)	*10.6*	*<0.01*	*n=22*

Table 2:

PCa patients	*BR* PSA (ng/ml)*	*BKV L-Tag mol testing*	*LTag serology (IgG)***	*Diagnostic PSA (ng/ml)*	*Gleason (score 1+2)*	*immune regulatory profiling****
#10	**0.2** (6wks)	+	+	5.50	6(3+3)	0.24
#17	**1.41** (6wks)	+	++	4.17	9(4+5)	0.12
#20	**0.31** (6wks)	+	++	4.50	7(3+4)	0.06
#21	**0.28** (6wks)	+	++	8.80	7(3+4)	0.20
#33	**0.2** (26wks)	+	+	*n.a*	6(3+3)	0.17

** Biochemical recurrence (BR) = PSA ≥ 0.2 ng/ml. Follow-up time in weeks (wks)*

*** IgG vs LTag (OD): (-) <0.04; (+) <0.1; (++) >0.1*

**** Cytokine gene expression upon LTag peptide-pool stimulation*

- *immune regulatory specific activity: ratio≤0.5*
- *proinflammatory specific activity: ratio≥1*
- *immune regulatory trend: 1≥ratio≥0.5*

Our findings thus suggest for a possible immune regulatory activity exerted by BKV L-Tag in PCa patients with poor clinical prognosis.

Achievements 2009

- The 104th Annual Meeting of the American-Urological-Association, Chicago, IL, APR 25-30, 2009
- The 2nd World Cancer Congress, Beijing, China, JUN 22-27, 2009

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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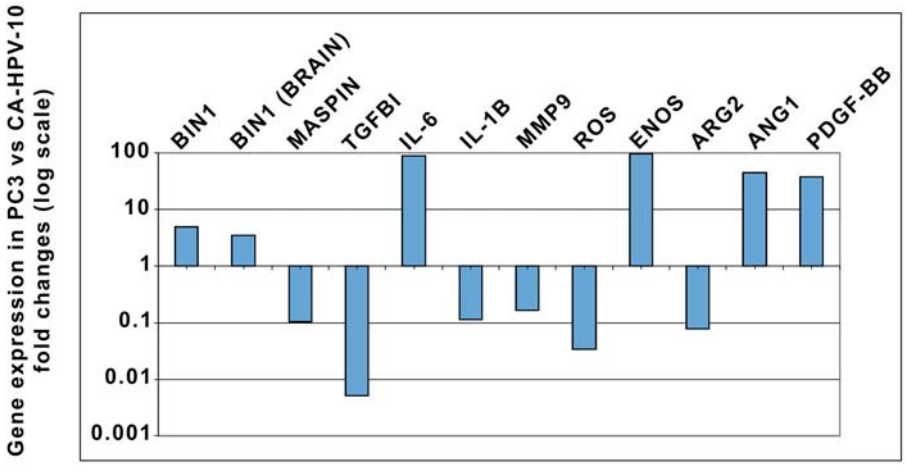
The role of inflammatory stimuli in modulating tumor derived factors expression in metastatic prostate cancer microenvironment

Banzola I, Mohyuddin R, Provenzano M

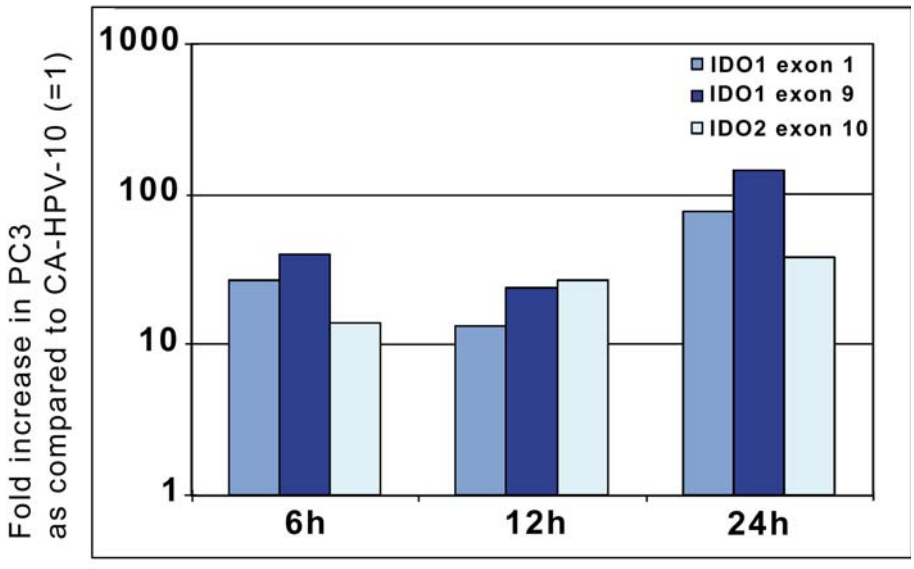
Tumor microenvironment is the battlefield were not well defined relationships between oncogenesis and immune surveillance to cancer take place. Upon transformation due to accumulation of several genetic mutations and epigenetic alterations, tumor cells proceed to either their full elimination or progression to overt cancer depending on immunity fitness. In particular, tumor immune escape occurs through the secretion of different tumor derived factors (TDFs) with immune suppressive properties, such as indoleamine, 2-3 dioxygenase (IDO), interleukin (IL) 10, transforming growth factor beta (TGF-β), and/or cytokines relevant in modulating the TDFs network. Among them, our interested focused on those microenvironmental modifiers that have been reported as possible mediators of prostate cancer (PCa) morbidity (IDO, IL-6, TGF-β). Two prostate cancer cell-lines with invasive (CA-HPV-10) and metastatic behaviour (PC3) were used. After culturing upon stimulation over 24 hours with IFN-γ and TNF-α, cells were harvested, total RNA extracted and transcribed into cDNA to perform gene expression by qrt-PCR. As housekeeping genes, either rRNA18S or β-actin was tested. A $2^{-\Delta\Delta Ct}$ method was used to compute fold changes among experimental conditions. A number of 35 genes accounted in either tumor immune escape or tumor progression were analysed and compared between CA-HPV-10 and PC3 at either constitutive or inducible level. They were divided in 4 groups: cytokines (IFN-γ, TGF- β, TNF-α, IL-1B, IL-2, IL-4, IL-6, IL-10) ; angiogenetic factors (VEGF-A, -C, -D, MMP2, MMP9, CCR7); immune tolerance factors (IDO-1, -2, ARG II, e.NOS, i-NOS, ROS); growth factors (ANG1 and 2, FRAG1, HGF, PDGF-BB). A fifth group of genes (miscellanea) was also included (cMYC, BIN1, Maspin, PSA, EpCAM, and TGFβI). Among those genes, 8 of them were not constitutively expressed in CA-HPV-10 (IFN-γ, IL-2, IL-4, IL-10, IDO-1, -2, HGF and PSA) and 4 of them in PC-3 (IFN-γ, IL-2, IL-4, and PSA). IDO-1, -2 and IL-10 were constitutively only expressed in bone metastases (PC3) than in localized tumor (CA-HPV-10) and the finding was inversely correlated to serine protease inhibitor maspin expression, while BIN1 and c-myc, relevantly expressed in both cell lines, were not modulated over time. When both cell lines were compared, a significant higher expression of IL-6, eNOS, ANG1, and PDGF-BB (above 20fold) was detected in PC3 while a significant higher expression of IL-1B, MMP9, ROS, and ARG2, was seen in CA-HPV-10. For cytokines like IL-2 and IL-4 gene expression was almost negligible (Figure 1).

Figure 1: Fold changes of most significant genes at constitutive level



Upon stimulation with IFN-γ (300U/ml), only an inducible higher amount of IDO-1 (500fold) and, at a lesser extent, of IDO-2 (20fold) was significantly detected in PC3 over the constitutive level. Conversely, a de novo expression for both IDO-1 and -2 was seen in CA-HPV-10. In particular, when we analyzed IDO enzyme gene expression in relation to its polymorphism (IDO-1 exon1 and exon9; IDO-2 exon1 and exon10) we found that the fold change previously seen between both cell lines was partially maintained for three genes (IDO-1 exon1, IDO-1 exon9, and IDO-2 exon10) at each time point used (6h: 50, 70, 20fold, respectively; 12h: 20, 40, 5fold, respectively; 24h: 90, 120, 60fold, respectively; Figure 2) while the expression for IDO-2 exon1 was almost negligible. .

Figure 2: IDO polymorphism gene expression fold changes



Conversely, the TNF-α treatment (300U/ml) produced a lower increase of expression for both IDO-1 exon1 and exon9 (15 and 20fold less, respectively) and IDO-2 exon10 (5-10fold less) in PC3, as compared to the expression seen upon IFN-γ stimulation, while there were no relevant variation in CA-HPV-10 upon both cytokine stimulation (data not shown). In addition, TNF-α stimulation induced a significant modulation for IL-1B, and MMP9 (Figure 3 and 4) in favor to localized tumor (CA-HPV-10) and IL-6 in favor to metastasis (PC3) over 24 hours (Figure 4). Particular attention deserves ROS gene, whose expression was constitutively higher in CA-HPV-10 cells and reverted into a significant expression in PC3 upon TNF-stimulation (Figure 4).

Figure 3: MMP9 modulation upon TNF-α induction over 24 hours

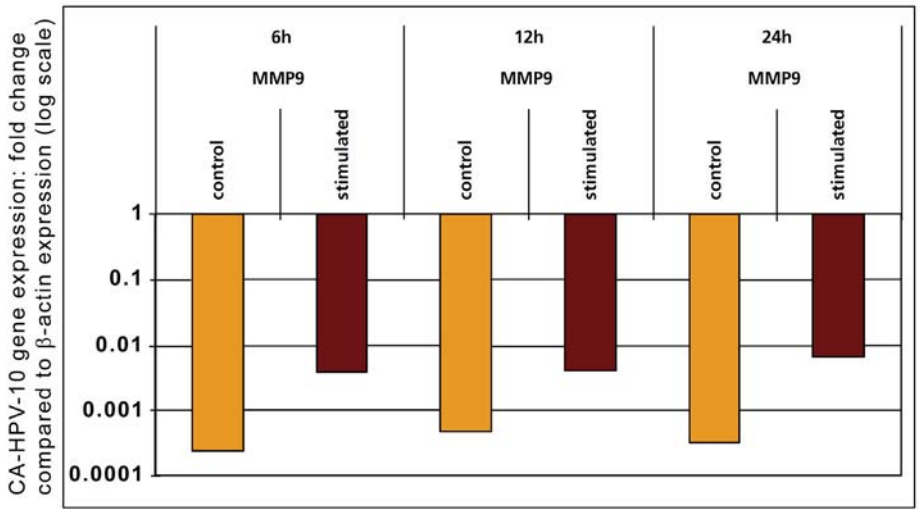
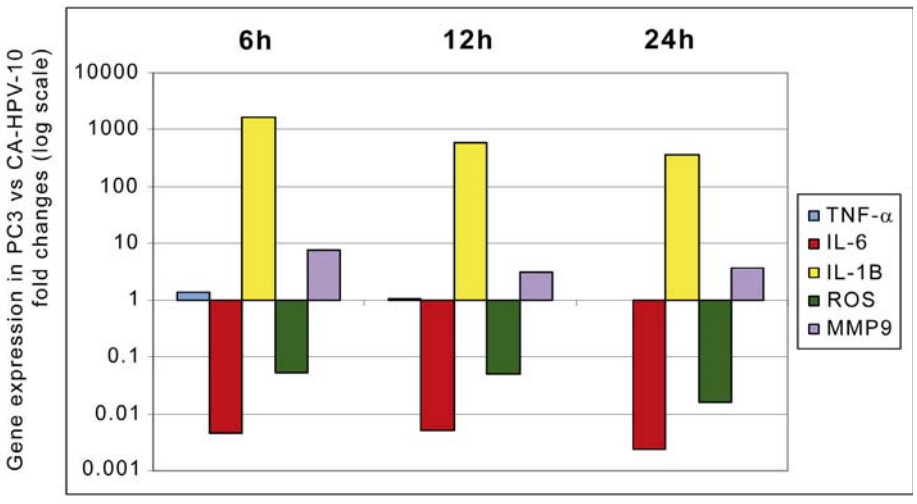


Figure 4: Fold changes of most significant genes upon TNF-α induction



Factors involved in immune suppressions such as IDO, IL-10, TGF-β and most likely mediators for PCa morbidity, also including IL-6, appear to be expressed at higher levels during PCa progression, in particular upon inflammatory stimuli. Moreover, our results point out on the possible implication of certain factors, such as MMP9, ROS and IL-1B in PCa progression and suggest for further specific investigations. An extended analysis on PCa patients might better inform on the role that inflammation plays in TDFs expression and activity.

Achievements 2009

- The 24th Annual Meeting of the International Society for Biology Therapy of Cancer, Washington DC, OCT 29-31, 2009
- The 2nd World Cancer Congress, Beijing, China, JUN 22-27, 2009

Collaborations:

- Molecular Tumour Pathology, Department for Surgical Pathology, University Hospital of Zürich.
- Department of Clinical Pharmacology, University of Florence, Italy.

Selected references:

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2.7.2 Tissue Engineering for Urologic Tissues

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Autologous Adult Stem Cell Therapy for Urinary Incontinence
Hefermehl L, Stölting M, Azzabi F, Sulser T, Eberli D

Urinary Incontinence is an involuntary leakage of urine occurring in up to one third of women. It can be caused by urinary sphincter injury due to congenital anomaly, trauma, surgery, child birth and ageing. Various treatment modalities have being performed, including surgery and injection therapies, with different outcomes. However, these therapy modalities fail to restore normal sphincter muscle function. Muscle bioengineering by using autologous muscle precursor cells (MPC) is proposed as a treatment option, in which cell transplantation of patients own cells would reconstruct functionally and morphologically the urinary sphincter. Research has been successfully performed in pre-clinical animal models by member of our group, and clinical translation presents as a following step. In order to allow rapid clinical translation culture conditions had to be optimized for human application. The optimal isolation and culture technique should be able to support cell growth and differentiation leading to formation of normal and functional human skeletal muscle.

Not only cell growth *in vitro*, but also muscle fiber formation and function *in vivo* were assessed in this study. MPCs were grown from human biopsies and expanded in culture on collagen coated dishes using DMEM medium enriched with insulin, dexamethason, human fibroblast growth factor and human basic endothelial growth factor. Detailed cell characterization using fluorescence-activated cell-sorting analysis and morphological analysis by fiber formation assay and Immunohistochemistry (IHC) at different passages were performed. Further, the applicability of these cells for tissue engineering purposes was assessed by measuring expansion potential, formation of myofibers and fused myotubes. Cells were implanted into the subcutaneous space of nude mice and engineered tissue retrieved after 2 and 4 weeks. We have established a culture technique for human MPCs that allows for reliable cell growth and expansion using collagen coated dishes and defined media only. Cell characterization demonstrated a muscle phenotype, the ability to form myofibers *in vitro* and *in vivo*. Tissue engineered muscle had not only muscle phenotype but also display muscle function verified by contractility upon electrical stimulation (figure 1).

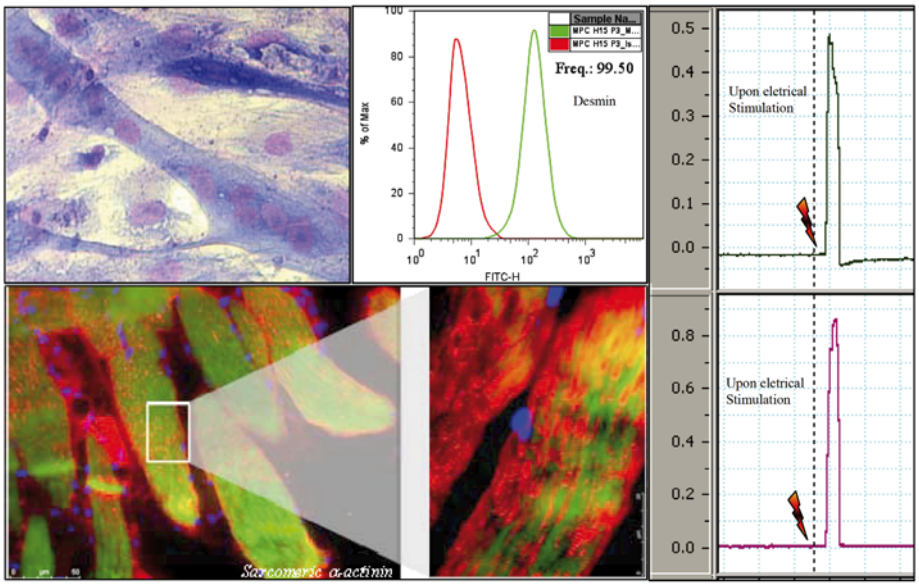

Figure 1 MPCs *in vitro* (top row) and Muscle formation *in vivo* (bottom row) after MPC injection. Top: MPC cell fusion and myofiber formation (Giemsa staining), MPC characterization by FACS analyses. Bottom: muscle tissue *in vivo* after dorsal subcutaneous injection in nude mice (α-actinin/Cy3, PKH64 marking injected muscle). Muscle contracts upon electrical stimulation.

Interactions of Adult Muscle Precursor Cells with Prostate Carcinoma: The safety of stem cell therapy of urinary incontinence in patients with previous prostate carcinoma.

Stölting M, Ferrari S, Sulser T, Becskei A, Handschin C, Eberli D

The replacement of terminally damaged organs remains a major problem in healthcare. The shortage of available donor organs and the high morbidity of immunosuppressive therapy lead to the application of regenerative medicine and tissue engineering to the field of organ replacement. The use of autologous cells and acellular or synthetic polymers for organ reconstruction has the potential to overcome these shortcomings and provide replacement organs made from the patients own cells. Muscle Precursor Cells (MPCs) are skeletal muscle cells sources capable of regenerating muscle fibers, and therefore investigated for the treatment of several muscular diseases. In Urology, it opens novel treatment possibilities including reconstruction of bladder muscles, management of sexual dysfunction and treatment of Urinary Incontinence.
In this study, we evaluate in vitro and in vivo the impact of MPC on different prostate carcinoma and sarcoma cell lines. In order to assess these bilateral interactions we determined growth rate, BIN1 expression (tumor suppressor expressed in differentiating muscle and absent in many tumors) and muscle differentiation, by FACS, immunocytochemistry and mRNA expression levels.

Differentiating muscle in the proximity of tumor caused a significant decrease ($p<0.001$) on cancer growth rate, induced cell cycle arrest and apoptosis (p21 and Caspase3 up-regulation) by reexpression of BIN1 and consequently by blocking c-Myc activity (figure 2). Conversely, muscle progenitor cell differentiation was significantly increased on the presence of tumor cells, inducing formation of well organized myotubes with all the features of normal and functional muscle. The results in vivo demonstrate tumor size decrease when injected with MPC (figure 3). Our data indicates that MPC downregulate tumor growth by induction of apoptosis, and is therefore a safe treatment for urinary incontinence of male patients after radical prostatectomy. The mechanisms involved on this interaction, tumor apoptosis and muscle differentiation are still under investigation.

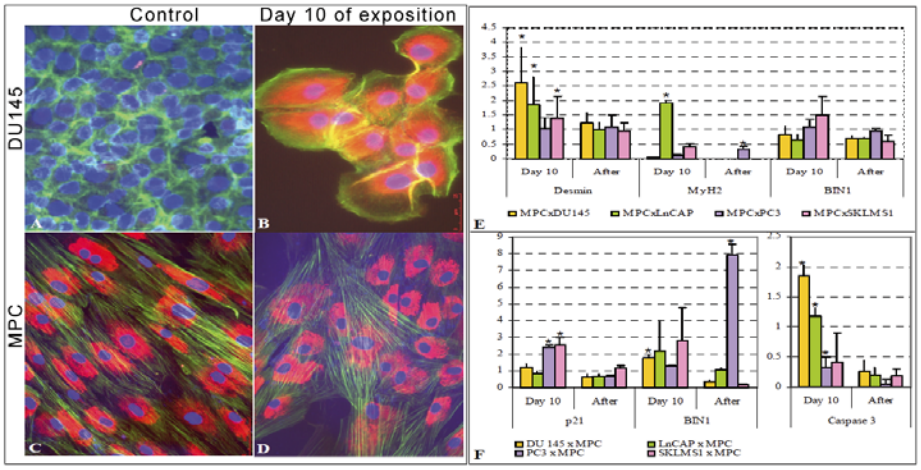

Figure 2 Cell characterization after exposition. ICC of Prostate carcinoma and MPC cells for BIN-1 before and after exposition, cytoskeleton labelled in green (Phalloidin 488) and secondary antibody in red (Cy3).
A) DU145 control after 10 days of culture; B) DU145 after 10 days in co-culture with MPC; C) BIN-1 expression on MPC control; D) BIN1 expression on MPC exposed to 10 days of co-culture with prostate carcinoma; E) RNA expression fold changes on desmin, Myosin Heavy Chain and BIN1on the 10th day of co-culture and 10 days after exposition. F) RNA expression fold changes on cancer cells expression of p21, BIN1 and Caspase 3. * $p < 0,05$

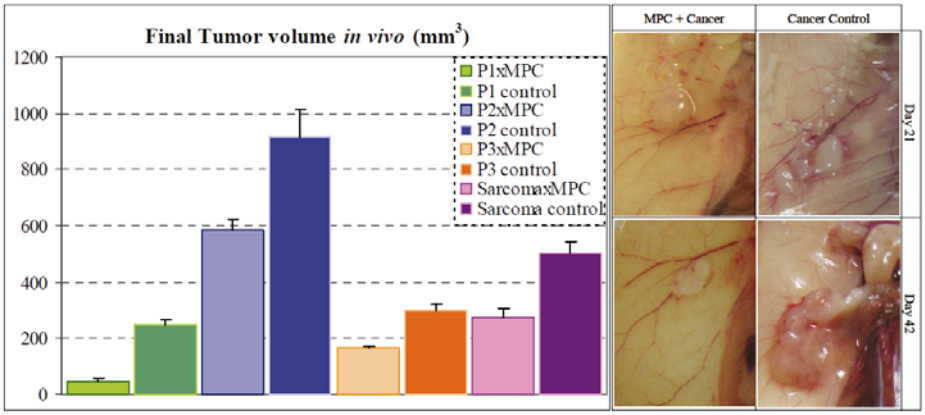

Figure 3 Tumor size *in vivo*. Prostate carcinoma cell according to metastasis potential (P1 = LnCAP; P2 = PC3, P3 = DU145). Final tumor volume after 42 days of implant – cancer on the presence of MPC demonstrated a lower volume then cancer injected alone. Three weeks after inplantation, the differences are already visible (Panel left) and after six weeks the final volumes can be evaluated on panel right.

Influence of harvest location on contractile function of engineered muscle tissue

Tremp M, Stölting M, Hefermehl L, Sulser T, Eberli D

Adult muscle precursor cells (MPCs) are envisioned as a cellular therapy for stress urinary incontinence. However, the results of recent animal studies and first human trials show a wide range of outcomes. The quality of cells used might be a key factor influencing muscle formation and functional outcome. In order to define which muscle would be most suitable for biopsy we investigated biopsies of predominately low twitch fibers, fast twitch fibers, weight bearing and non-weight bearing muscles. Biopsies were taken, characterized and functionally investigated in vivo. Muscle biopsies from the quadriceps, rectus and soleus muscle of Lewis rats were obtained, histologically analysed and MPCs expanded in culture. Precursor cell density (PAX7) and myofiber type was determined by immunohistochemistry and histomorphometry. Cell expansion potential was evaluated by proliferation assays and compared. MPCs of each group were characterized by FACS analysis (Desmin, MyoD and MHC). In vivo muscle tissue formation and function was assessed by injecting MPCs s.c. into nude mice. After 2 weeks gross examination, immunohistochemistry and functional organ-bath studies (40V/32Hz) of the engineered muscles were performed.

Our preliminary results demonstrate by histomorphometric analysis a high expression of PAX7 positive cells in the soleus muscle (23.9% ± 7.4%), whereas the expression was lower in the rectus muscle (18.8% ± 4.8%) and quadriceps muscle (12.4% ± 0.8%). The doubling time in the exponential phase of the growth curve from P1 – 5 of the soleus MPCs was 29.6 ± 5.75hours, of the rectus MPCs 37.01 ± 3.2hours and of the quadriceps MPCs 40.9 ± 7.8 hours. We were successful in engineering muscle tissue of all sources. At harvest, the weight of the engineered muscles of the soleus group was 53.15mg ± 11.45mg, whereas the weight of the quadriceps and rectus group was lower (37.2mg ± 2.4mg and 30.5 ± 6.4mg, respectively). The same trend was seen in the functional organ-bath studies on Electrical Field Stimulation (EFS) at 40V and 32Hz with the highest contraction per 100mg tissue of the soleus MPCs (671mg ± 47.2mg), whereas the rectus MPCs (433mg ± 255mg) and quadriceps MPCs (183mg ± 98mg) had a lower contraction (Figure 4).

In this study we demonstrated that the origin of the muscle sample taken has a significant impact on muscle tissue engineering with differences in expansion potential, tissue formation and muscle contraction. The presented data suggest that MPCs harvested from low twitch and weight bearing muscles (soleus) will lead to the best functional outcome.

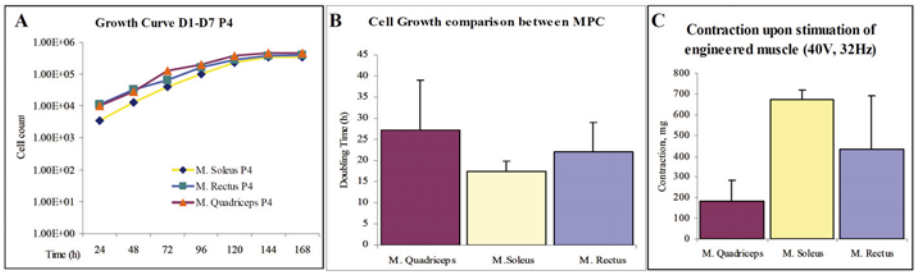

Figure 4 Cell proliferation assay of myoblasts isolate from the soleus, quadriceps and rectus abdominis muscle at passage 4 (A). Cells were plated at 3x103 cells/cm2 and mononucleated cells were counted after trypsinization. Results are mean ± SEM of three experiments per mg. Myoblasts from the soleus muscle had a faster growth rate than those from the quadriceps or rectus abdominis muscle from P1 – P5 (B). Functional organbath analysis of engineered muscle on EFS at 40V and 32Hz (C). The maximum tension was recorded and normalized to the sample weight (mg/100mg tissue). The data showed that functional muscles were engineered from cells of all three muscle types with the highest contraction from the soleus muscle. *Error bars* represent standard error.

Impact of neurorehabilitation after spinal cord injury on bladder function

Horst M, Madduri S, van den Brand R, Musienko P, Gobet R, Gratzke C, Sulser T, Courtine G, Eberli D

Spinal cord injury (SCI) not only induces paralysis, but also leads to bowel and bladder dysfunction. After complete spinal cord transections, removing all supraspinal inputs in adult rats, it has been demonstrated that combinations of locomotor training, pharmacological and epidural electrical stimulation interventions can remodel spinal circuits, leading to significant recovery of walking and even running in paralyzed rats. The potential of these interventions to improve bladder function have not been studied. We therefore investigate weather neurohabilitation can positively effect bladder function after severe spinal cord injury. In this study, fourteen adult Lewis rats received staggered thoracic lateral hemisections interrupting all direct supraspinal inputs. Rats were classified into two groups: trained and untrained. 5 healthy animals served as normal control. At 8 weeks post SCI, bladder function was evaluated by measuring residual bladder volume and organbath studies including electrical field stimulation and pharmacological tests. To asses the effect of neurorehabilitation on the urinary system, blood samples were taken and analysed for creatinine and cystatin C. Kidneys and bladder were extracted for morphological, functional ,histological and immunohistochemical investigations.
Our results indicate that function, size of kidney and renal pelvis were comparable between the three groups. All animals with SCI showed increased bladder size, volume and wall thickness compared to healthy rats ($p < 0.05$). The ratio between connective tissue and smooth muscle of the bladder wall was decreased ($p < 0.05$). Immunostaining with neuropeptide Y revealed decreased efferent innervation in rats with SCI. However, no significant differences between trained and untrained rats could be detected for any of these measures. Neurofilament 200 positive afferent nerve fibers (A fibers), were increased whereas untrained animals showed a markedly higher density a A fibers than trained rats.

Pharmocological stimulation in the organ bath with carbachol and ATP did not show any significant difference between the groups. Nevertheless, higher residual volumes ($p < 0.05$) and increased response to electricial field stimulation were observed in trained compared to untrained rats.
Although neurorehabilitation can dramatically improve stepping capacities following a severe SCI in adult rats, our preliminary data could not demonstrate a beneficial effect on bladder function. However there are some differences in the innervation pattern and excitability in trained compared to untrained rats. Stimulations can promote significant benefits for functional recovery including walking and even running in paralyzed rats. The potential of these interventions to improve bladder function have not been studied. We therefore investigated, weather neurohabilitation can positively effect bladder function after severe spinal cord injury.
To compare the distribution pattern of afferent and efferent innervation in the bladder wall in the three groups, we need to complete the immunhistochemical staining with the above mentioned specific antibodies. We already established protocols for each antibody for positive control (sciatic nerve), but the methods need to be adapted and the technique optimized specifically for bladder tissue. Although neurorehabilitation following complete transection of the spinal cord in adult rats can improve stepping capacities its beneficial effect on bladder function could not be demonstrated so far with our investigation.

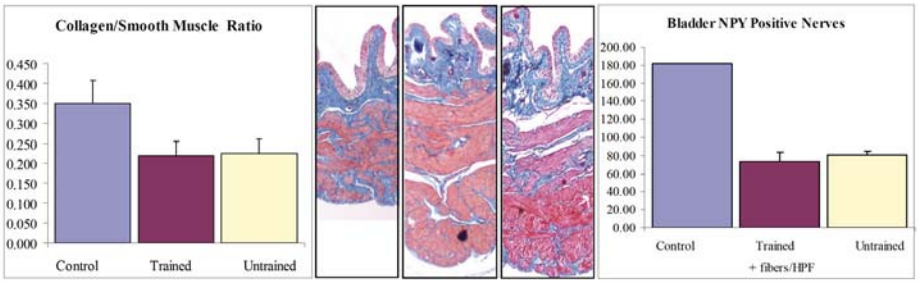

Figure 5 The ratio between connective tissue and smooth muscle of the bladder wall was decreased ($p < 0.005$) compared to healthy rats. But there was no difference between trained and untrained animals. Immunostaining with neuropeptide Y (NPY) revealed decreased efferent innervation. However, no differences between trained and untrained animals could be detected.

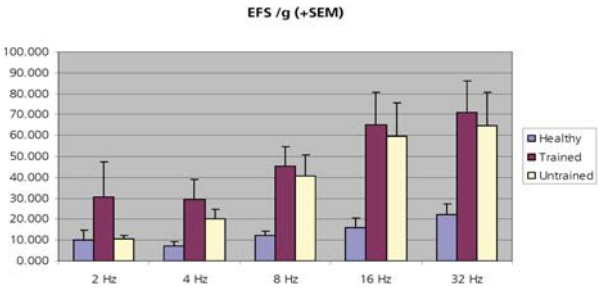

Figure 6 Organ bath study: Electrical field stimulation, showed increased contractile force in trained compared to untrained rats. These results were not statistically significant, potentially due to the small number of samples. However, this could possibly be caused by hypercontractility.

In vivo MRI Imaging of Adult Stem Cells

Azzabi F, Njiwa J, Sulser T, Rudin M, Eberli D

Tracking transplanted cells in a non-invasive manner allows us to investigate homing process, distribution, local retention and functional integration of transplanted stem cells.
The ability to monitor these cells in “real time” would allow us to further improve the transplantation procedures and effectiveness of the cell-based therapies. Different imaging techniques are in use for cell tracking. Magnetic resonance imaging (MRI), nuclear imaging and optical imaging are the most commonly investigated. MRI is a strong diagnostic technology that provides detailed morphologic and structural information and is efficient in visualizing labeled cells. Superparamagnetic iron oxide (SPIO) particles were first employed as contrast agent for organs. Recent research has shown that they can also be incorporated into stem cells prior to transplantation and provide an elegant manner to track them in vivo by MRI imaging. The intensity and location of the signal allows drawing conclusions on survival and locomotion of the injected cells: if cells die after injection the beads are removed by macrophages leading to a reduced signal but if cells divide the beads get shared, however the signal remains at the same location. The SPIO labeling coupled with MRI was used successfully with other types of stem cells in different tissues showing no alteration in proliferation and differentiation potential. However, this technology was never used to label MPCs. Therefore, in this study, we will investigate the integration of iron oxide labeled human MPCs into host tissue by following their migration and integration to the damaged muscle. Further we will evaluate the effect on myogenic differentiation and functional properties. If successful, this method would not only allow us to better understand the cellular processes after injection of stem cells in vivo but also reduce the number of animals used for this research.
In order to obtain MRI detectable cells Iron oxide (SPIO) particles are inserted to animals used for this research. Our preliminary data show that we have been successful to label the MPC and that injected cells could be detected my MRI (figure 7).

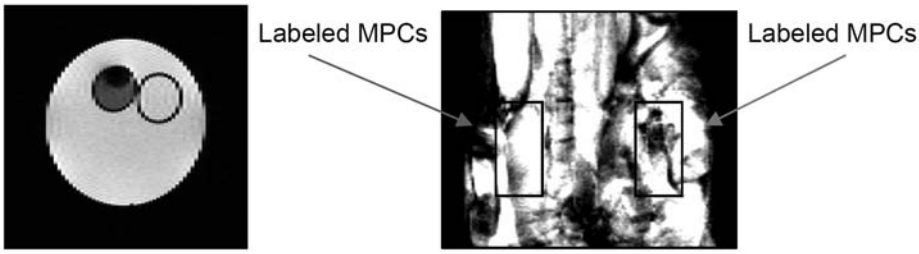

Figure 7 *In vitro* and *in vivo* MRI scanning of MPCs: A) MPCs have been concentrated in eppendorfs. On the right side the dark spot in the eppendorf represents the signal by MPCs with iron oxide. On the left side, it is the control. B) MPCs have been injected subcutaneously with a collagen carrier into both back sides of a death mouse: On the right side, the MPCs are labeled with iron oxide and on the left side, MPCs are injected without iron oxides.

Achievements 2009

Grants:

- Institutional Research Grant from Eleonoren-Stiftung and Openheimer Investments. Innervation of Tissue Engineered Bladder Constructs for Functional Reconstruction
- Research Grant from “SNF, Schweizer National Fonds“ 323230_126230/1. For research project: “Adult Muscle Progenitor Cells for the Treatment of Urinary Incontinence”
- Matching Fund, Research Committee, Medical School Zurich. For research project: “Adult Muscle Progenitor Cells for the Treatment of Urinary Incontinence”.
- Research Grant form EMDO Stiftung, For research project: “Adult Muscle Progenitor Cells for Clinical Applications: Function, Safety and Interactions”, Meline Stölting M.D.
- Research Grant from “Stiftung für Forschung an der Medizinischen Fakultät“. For research project: “Adult Muscle Progenitor Cells for Clinival Applications: Function, Safety and Interactions,” Meline Stölting M.D.
- Research Grant from “Fonds für Medizinische Forschung“, For research project: “Adult stern cells for the treatment of bladder hypocontractility,” Mathias Tremp M.D.
- Research Grant from “Max & Hedwig Niedermayer Stifung“, Research project: “Adult stern cells for the treatment of bladder hypocontractility,” M.Tremp M.D.

Prizes/Awards:

- Prize of the Swiss Association of Urology. For the research work performed in the field of urologic tissue engineering. Annual Meeting of the Schweizerischen Gesellschaft für Urologie, Lausanne
- Posterprize: Best Poster of the Session of the European Association of Urology. Eberli D, Hefermehl LJ, Sulser T, Knönagel H. Lateral temperature spread of vessel-sealing devices: Are they safe for nerve sparing radical prostatectomy? Annual meeting of the European Association of Urology, Stockholm, 2009
- Posterprize: Best Poster of the Session Kongress der Deutschen Gesellschaft für Urologie. Hefermehl LJ, Eberli D, Sulser T, Knönagel H. Lateral temperature spread of vessel-sealing devices: Are they safe for nerve sparing radical prostatectomy? Kongress der Deutschen Gesellschaft für Urologie, Düsseldorf , 2009

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- Prof. James Yoo, Wake Forest University School of Medicine, Medical Center Boulevard, Winston-Salem.
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- Prof. Janos Vörös, ETH-Zentrum, Institut f. Biomedizinische Technik.
- PD Dr. med. Caroline Maake, University of Zürich, Institute of Anatomy, Zürich, Switzerland.

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- Horst M, Madduri S, Gobet R, Maduri S, Sulser T, Hall H, Eberli D. Scaffold Characteristics for Functional Hollow Organ regeneration. Advances in Biomaterials, Materials, 2010, 3, 241-263 ISSN 1996-1944

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- Eberli D, Hefermehl LJ, Sulser T, Knönagel H. Lateral temperature spread of vessel-sealing devices: Are they safe for nerve sparing radical prostatectomy? Eur Urol Suppl 2009
- Hefermehl LJ, Sulser T, Knönagel H. Lateral temperature spread of vessel-sealing devices: Are they safe for nerve sparing radical prostatectomy? J Urol 2009.
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- L.J. Hefermehl, M. Stölting, F. Azzabi, T. Sulser, D. Eberli. Optimization of Human Skeletal Muscle Precursor Cell Couluter and Myofiber Formation for Sphincter Reconstruction. Kongress der Schweizerischen Gesellschaft für Urologie, Lausanne (2009)
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- Eberli D, Hefermehl LJ, Sulser T, Knönagel H. Lateral temperature spread of vessel-sealing devices: Are they safe for nerve sparing radical prostatectomy? Kongress der Deutschen Gesellschaft für Urologie, Düsseldorf (2009)

2.7.3 Erectile Dysfunction

Dr. med.
Alexander Müller

Penile Rehabilitation after Radical Prostatectomy:
The cavernous nerve crush injury model in the rat
Müller A

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.

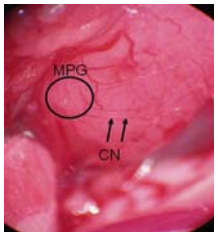

Figure 1:
Cavernous Nerve in the Rat Intra-operative picture showing the cavernous nerve (CN) coming from the major pelvic ganglion (MPG) and running along the surface of the prostate in the rat.

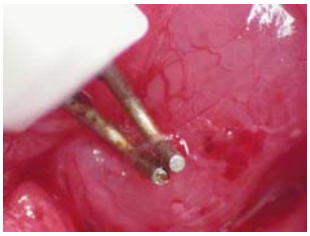

Figure 2:
Intra-operative picture displaying the cavernous nerve (CN) hooked up by an electrode for electrical stimulation to measure the maximum intracavernosal pressure (ICP). At the same time of CN simulation the corresponding mean arterial blood pressure (MAP) will be reported as the ICP/MAP ratio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

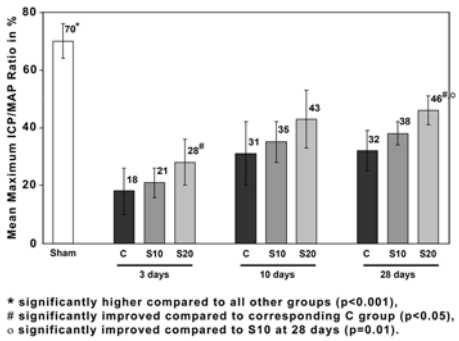

Figure 3: Functional Results
Graph showing the mean maximum intracavernosal pressure (ICP) divided by the corresponding mean arterial pressure (MAP), reported as ICP/MAP ratio as a percentage for Control (bilateral CN crush), and both treatment groups S10 (daily 10 mg/kg sildenafil sc) and S20 (daily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

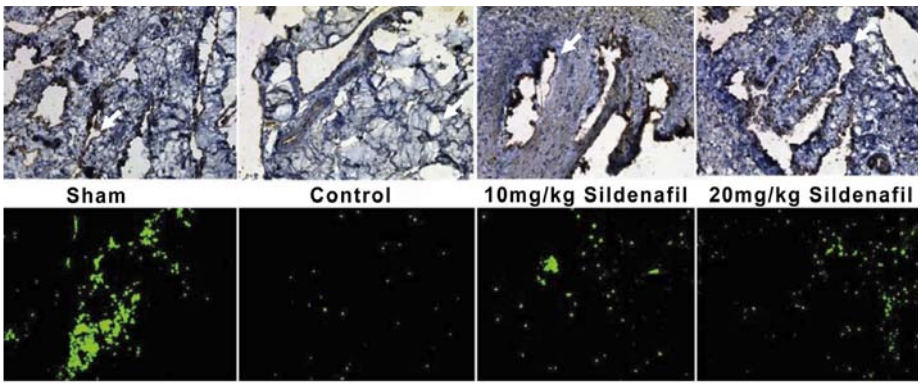

Figure 4: Immunohistochemistry staining for CD31
At 28 days after cavernous nerve injury the Control group (bilateral CN crush) demonstrated a lower density of CD31 staining compared to Sham (no CN crush) and both treatment groups S10 und S20 displayed higher staining compared to control on both immunohistochemistry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

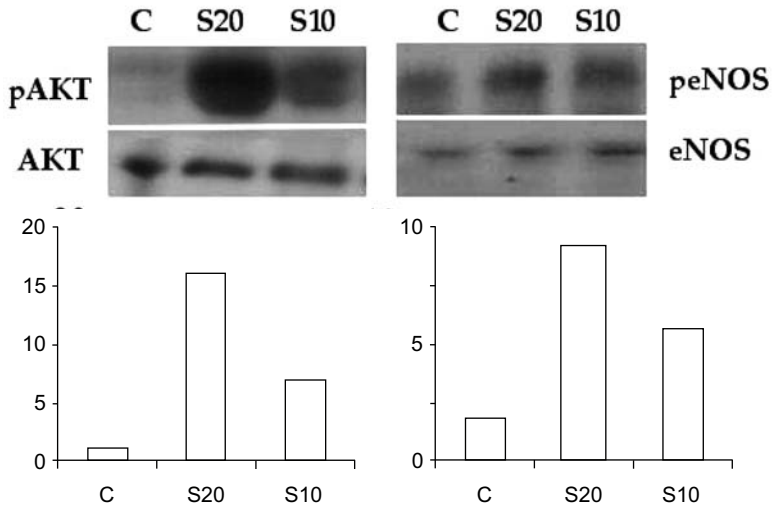

Figure 5: Immunoblotting for eNOS/AKT
Both treatment groups S10 and S20 (10 and 20 mg/kg sildenafil sc daily) demonstrated greater activation (phosphorylation) of AKT and eNOS compared to the Control group C.

Achievements 2009

2. Book Chapter

- Pharmacologic Penile Preservation and Rehabilitation
  Alexander Muller and John P. Mulhall
  Departments of Urology at Memorial Sloan Kettering Cancer Center & Weill Medical College of Cornell University, New York, NY, USA
  In Sexual Function in the Prostate Cancer Patient, 1st Edition by Mulhall
  Humana Press, Publication 2009

Nationale und Internationale Kongressbeiträge

- 61. Kongress der Deutschen Gesellschaft für Urologie e.V., 17-19. September 2009, Dresden, Deutschland
- Can intracavernosal injection therapy salvage PDE5 inhibitor failures?
  Müller A, Tal R, Mulhall JP
- Microarray analysis of the impact of sildenafil treatment on gene expression in the rat cavernous nerve injury model. Müller A, Kobylarz K, Mulhall JP
- Apoptotic protein profiling of cavernosal tissue following cavernous nerve injury. Müller A, Kobylarz K, Mulhall JP
- The development of an in vivo model for the assessment of cigarette smoking-associated erectile dysfunction
  Müller A, De Lorenzo M, Kobylaz K, Dannenberg A, Mulhall JP
- Predictors of venous leak development in men following radical prostatectomy. Muller A, Rojas Cruz C, Tal R, Choi JM, Nelson C, Mulhall JP
- Assessment of the use of sildenafil citrate for the protection of erectile tissue and function following castration in the rat model
  Müller A, Deveci S, Kobylarz K, Tal R, Sulser T, Mulhall J
- Das onkologische Outcome nach retroperitoneoskopischer Tumornephrektomie im Verlgich zur offenen Tumornephrektomie beim lokalisierten Nierenzellakarzinom ≤ 7 cm
  Hermanns T, Strebel RT, Müller G, Müntener M, Weltzien B, Müller A, Rufibach K, Schmid DM, Seifert HH, Bachmann A, Sulser T, Wyler S

65. Jahrestagung der Schweizerischen Gesellschaft für Urologie, 3-5 September 2009, Lausanne, Schweiz

- Can intracavernosal injection therapy salvage PDE5 inhibitor failures?
  Müller A, Tal R, Sulser T, Mulhall JP
- Apoptotic protein profiling of cavernosal tissue following cavernous nerve injury. Müller A, Kobylarz K, Sulser T, Mulhall JP
- The development of an in vivo model for the assessment of cigarette smoking-associated erectile dysfunction
  Müller A, De Lorenzo M, Kobylaz K, Sulser T, Dannenberg A, Mulhall JP
- Assessment of the use of sildenafil citrate for the protection of erectile tissue and function following castration in the rat model
  Müller A, Deveci S, Kobylarz K, Tal R, Sulser T, Mulhall J
- Rezidiv einer partiellen einseitigen Schwellkörperthrombose nach Absetzten der Oralen Antikoagulation.
  Tremp M, Seifert H-H, Sulser T, Müller A

24th Annual EAU Congress, 17-21 March 2009, Stockholm, Sweden

- The development of an in vivo model for the assessment of cigarette smoking-associated erectile Dysfunction (Best Poster Award)
  Müller A, De Lorenzo M, Kobylaz K, Dannenberg A, Mulhall JP
- Transurethral plasma vaporization of the prostate in saline (TUViS-P) in patients under continuing oral anticoagulation.
  Müller A, Bigger KDP, Blick N, Suter S

Best Poster
24th Annual EAU Congress, 17-21 March 2009, Stockholm, Sweden

- The development of an in vivo model for the assessment of cigarette smoking-associated erectile dysfunction
  Müller A, De Lorenzo M, Kobylaz K, Dannenberg A, Mulhall JP

Best Poster
61. Kongress der Deutschen Gesellschaft für Urologie e.V.,
17-19. September 2009, Dresden, Deutschland

- The development of an in vivo model for the assessment of cigarette smoking-associated erectile dysfunction
  Müller A, De Lorenzo M, Kobylaz K, Dannenberg A, Mulhall JP
- Assessment of the use of sildenafil citrate for the protection of erectile tissue and function following castration in the rat model
  Müller A, Deveci S, Kobylarz K, Tal R, Sulser T, Mulhall J

SGU-Poster Price
65. Jahrestagung der Schweizerischen Gesellschaft für Urologie,
3-5 September 2009, Lausanne, Schweiz

- Apoptotic protein profiling of cavernosal tissue following cavernous nerve injury. Müller A, Kobylarz K, Mulhall JP
  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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  A multi-institutional evaluation of active surveillance for low-risk prostate cancer.
  J Urol. 2009 Apr;181(4):1635-41; discussion 1641. Epub 2009 Feb 23.
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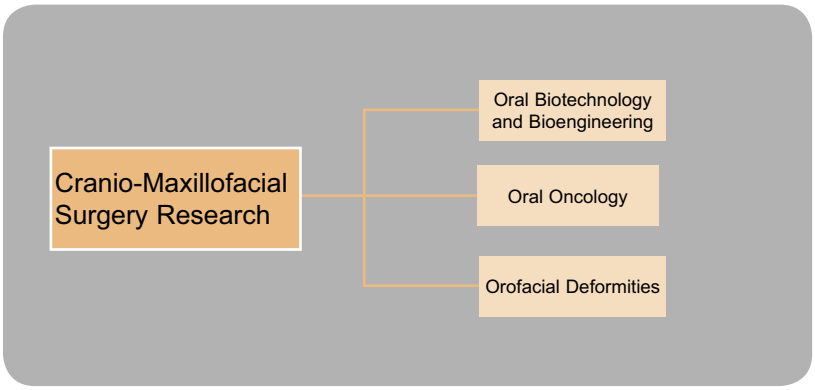
  The correlation between intracavernosl pressure and cavernosal blood oxygenation
  J Sex Med. 2009 Oct;6(10):2722-7. Epub 2009 Aug 4

2.8 Cranio-Maxillofacial Surgery Research

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

Prof. Dr. Dr.
Klaus W.Grätz



2.8.1 Oral Biotechnology and Bioengineering

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PD Dr. rer. nat.
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Martin Ehrbar
PhD

Katrin Lange
PhD

Ana Sala
PhD-student

Rita Correro
PhD-student

Patrick Hänseler
PhD-student

Alexander
Tchouboukov

Yvonne
Bloemhard

BMP and bone regeneration
Weber F, Ehrbar M, Lange K, Sala A, Correro R,
Hänseler P, Tchouboukov A, Bloemhard Y

Surgeons are in an ongoing search for the ideal solution of bridging larger bony defects in the facial skeleton. In recent years with the advent of microsurgical techniques major strides towards better long term outcomes have been made. The solutions however are a mere manipulation and adaption of bony tissue from another area to more or less fit the requirements. Morbidity and costs are major prohibitive factors in this regard. Free bone grafting as an alternative is notoriously unpredictable for larger defects with infection and unpredictable resorption being the predominant culprits. A dire need exists for a custom made, functional, stable and reliable bone mass that can replace a native mandible or maxilla segment. Pre-planning of future implants is an essential need ensuring adequate future function and quality of life for our patients. The identification of the bone inducing principle was a major step towards solving these problems. But to date we are still not able to deliver the bone morphogenetic proteins (BMPs), responsible for the bone inducing principle, in combination with a bone substitute material for challenging applications, like the substitution of a mandible. Therefore we work on the development of optimal delivery systems for BMPs and other growth factors needed for predictable and reliable bone regeneration in the clinic. One obstacle in the routine use of growth factors in the clinic is associated with high cost. In the last years we identified and characterized enhancers for BMPs able to half the amount of BMP needed in clinical applications. At the moment we try to develop double delivery systems for growth factors and their enhancers to make good use of this combination for bone regeneration purposes.

Synthetic hydrogels
One possibility to deliver BMPs is to use an artificial matrix which will only contain the cues necessary for the formation of a vascularized bone.

In the last years we developed a fully synthetic, fibrin-like matrix material mainly composed of polyethylenglycol (PEG). Due to its simplicity in terms of biological cues, this material can be used to study the effect of single components added to the material on bone formation in vivo and in vitro. In addition it can also serve as BMP delivery matrix. Here we developed new strategies to link BMPs to the matrix covalently.

Bone substitute materials

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

1) Synthetic hydroxyapatite/tricalciumphosphates (HA/TCP).

The goal of this subproject is the characterization and development of synthetic HA/TCP based materials with emphasis on the attachment and release of growth factors.

2) Synthetic and natural hydrogels

Hydrogels are ideal ingrowth matrices for regeneration purposes but their mechanical properties are insufficient for bone regeneration purposes. Therefore we want to combine hydrogels with mechanically more stable materials like hydroxyapatite to form novel bone substitute composites.

3) Porous bioactive glass

“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. 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.

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.

2.8.2 Oral Oncology

Dr. med
Astrid Kruse

Dr. med.
Marius Bredell

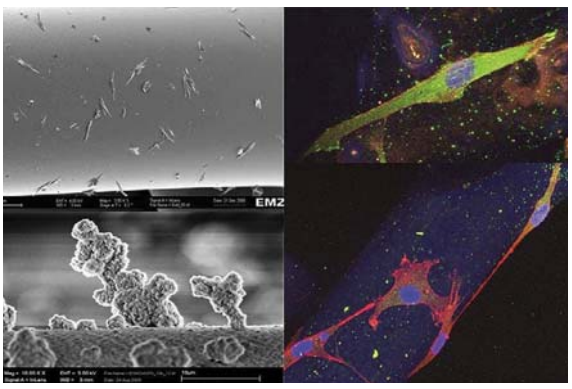
The estimated number of newly diagnosed cancers of the oral cavity and pharynx is 97.800 patients per year; the estimated number of deaths due to these carcinomas in Europe is 40.100 per year. Metastasis is thought to be responsible directly or indirectly for more than 90% of all cancer deaths.
Despite improved diagnostic tools, chemotherapy, radiotherapy and improved surgical techniques, the five-year survival rate for head and neck cancer persists at a very low 50 % level.
Despite the fact that all these cancers belong to the category of head and neck cancers, their clinical features and progression can be very different. While some tumours have a low tendency to malignancy, others infiltrate in a very early stage, show lymphoangiosis carcinomatosis or perineural infiltration, irrespective of the patient being exposed to well known risk factors like smoking, alcohol abuse, bad mouth hygiene or HPV. The question why in some cases tumours do have such a progressive outcome while others seems to have a slow tendency of infiltration is still not answered. Therefore we started to evaluate the clinical risk factors in patients with head and neck carcinoma and extend these studies now by an immuno-histochemical dataset generated from paraffin embedded sections of head and neck cancer to link them to clinical parameters and radiological findings.

2.8.3 Orofacial Deformities

PD Dr. med
Joachim Obwegeser

Dr. med.
Christine Jacobsen

Until today, several standardized therapy methods for the surgical correction of craniofacial deformities were developed. In some severe cases, surgical distraction of syndromic craniofacial deformities is the method of choice to achieve the best possible functional and aesthetic result.
During the last decade different devices for surgical distraction in the facial area were developed, but all of them with disadvantages of difficulty in correct vector determination. Additionally some devices show early loosening and unaesthetic scarring, especially in small children. For this a new external distraction device for distraction in Le Fort III level was developed in collaboration with KLS Martin Group. This device was successfully inserted in a child with syndromic craniosynostosis.



Surface treated bioactive glass scaffold for tissue engineering and the cellular response (vinculin green, actin red).

Achievements 2009

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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, Dr. Martin Ehrbar))
- ETH Zurich, Laboratory of Biosensors and Bioelectronic (Prof. Janos Vörös)
- ETH Zurich, Department of Materials (Prof Marcus Textor, PD Dr. Heike Hall-Bolic, Prof. Viola Vogel)
- 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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2.9. Surgical Intensive Care Medicine

PD Dr. med.
John F. Stover

Prof. Dr.
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Dr. med.
Markus Béchir

Mario Fasshauer

Giovanna Brandi

Jutta Sommerfeld

Dr. med.
Reto Schüpbach

Angela Fendel

Search for optimal hematocrit value during the early phase following severe traumatic brain injury

Following severe traumatic brain injury (TBI) the optimal hematocrit during the initial operating room (OR) phase is discussed controversially. We hypothesized that hematocrit values exceeding 28%, the local hematocrit target reached by the end of the initial OR phase, resulted in more complications, increased mortality, and impaired recovery compared to patients in whom hematocrit levels did not exceed 28%.
To investigate the Impact of hematocrit (independent variable) reached by the end of the OR phase on mortality and morbidity determined by the extended Glasgow outcome scale (eGOS; dependent variables), a retrospective analysis was performed in 139 TBI patients. In addition, multiple logistic regression analysis was performed to identify additional important variables.

Main results
Following severe TBI, mortality and morbidity were neither aggravated by hematocrit above 28% reached by the end of the OR phase nor worsened by the required transfusions. Upon multiple logistic regression analysis, eGOS was significantly influenced by the highest intracranial pressure and the lowest cerebral perfusion pressure values during the initial OR phase reflecting the severity of the underlying brain damage.

Conclusions
Based on this retrospective observational analysis, increasing hematocrit above 28% during the initial OR phase following severe TBI was not associated with improved or worsened outcome. This questions the need for aggressive transfusion management. Prospective analysis is required to determine the lowest acceptable hematocrit value during the OR phase which neither increases mortality nor impairs recovery. For this, a larger caseload and early monitoring of cerebral metabolism and oxygenation are indispensable.

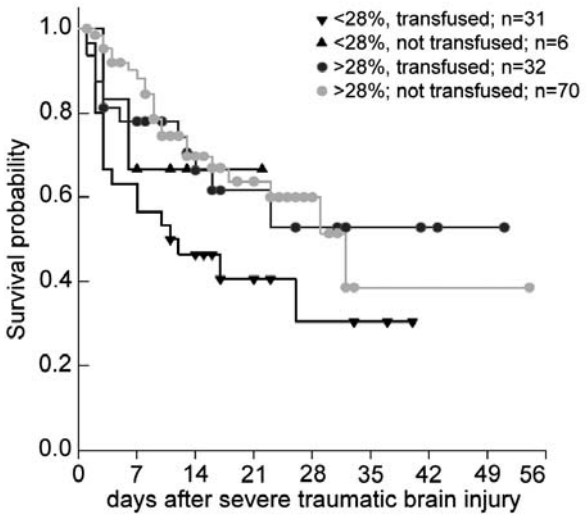

Figure 1
Impact of hematocrit target >28% on survival in transfused and not transfused TBI patients. Despite a trend to increased mortality in transfused patients not reaching the pre-defined hematocrit target > 28%, there was no statistically significant difference.

Feasibility of color coded transcranial Duplex sonography in assessing intracranial pressure and cerebral perfusion pressure non-invasively following severe traumatic brain injury

According to different authors intracranial pressure (ICP) and cerebral perfusion pressure (CPP) can be estimated non- invasively using transcranial color coded duplex sonography (TCCDS).
Accuracy and identification of the optimal equation was performed in an observational clinical study in a total of 45 continuously sedated (BIS< 50), normoventilated (paCO2> 35 mmHg), and non-febrile patients with severe traumatic brain injury (TBI).

Methods
Estimated ICP (eICP) and estimated CPP (eCPP) based on TCCDS- derived flow velocities and arterial blood pressure values using three different equations were compared to actually measured ICP and CPP in severe TBI patients. Optimal equation was assessed by Bland- Altman analysis.

Results
The equations: ICP= 10.927 X PI (pulsatility index)– 1.284 and CPP= 89.646 – 8.258 x PI resulted in eICP and eCPP similar to actually measured ICP and CPP with eICP 10.6 ± 4.8 vs. ICP 10.3 ± 2.8 and eCPP 81.1 ± 7.9 vs. CPP 80.9 ± 2.1 mmHg, respectively.
The other two equations eCPP= (MABP x EDV)/ mFV + 14 and eCPP= [mFV/ (mFV- EDV)] x (MABP- RRdiast) resulted in significantly decreased eCPP values: 72.9 ± 10.1 mmHg and 67 ± 19.5 mmHg, respectively. Superiority of the first equation was confirmed by Bland- Altman revealing smallest standard deviations for eCPP and eICP.

Conclusions
TCCDS- based equation (ICP= 10.927 X PI – 1.284) allows to screen patients at risk of increased ICP and decreased CPP. However, adequate therapeutic interventions need to be based on continuously determined ICP and CPP values.

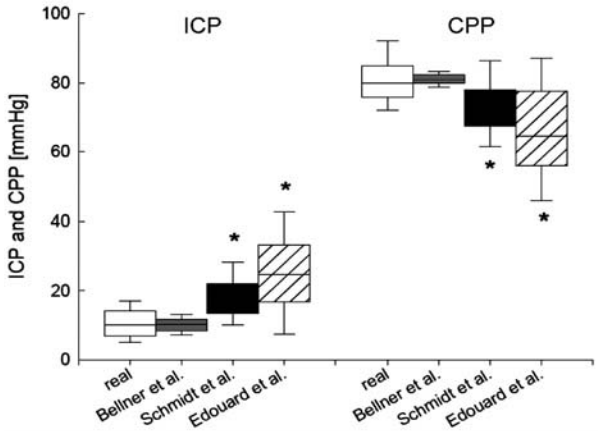

Figure 2
Estimated ICP and CPP determined by the equation published by Bellner et al. were similar to the actually measured ICP and CPP values. Worst accuracy was obtained with the other two equations (Schmidt et al. and Edouard et al.).

Evaluation of non-invasive cardiac output measurement in hemodynamically unstable critically ill patients

Monitoring of cardiac output and blood pressure are standard procedures in critical care medicine. Traditionally, invasive techniques like pulmonary artery catheter (PAC) and arterial catheters are widely used. However, their invasiveness bears many risks of deleterious complications. Therefore, a noninvasive reliable cardiac output (CO) and blood pressure monitoring system could improve the safety of cardiac monitoring. The aim of the present study was to compare a noninvasive versus a standard invasive cardiovascular monitoring system in critically ill patients.

For this, Nexfin HD was compared to a pulmonary artery catheter and regular arterial catheter. Nexfin HD is a continuous noninvasive blood pressure and cardiac output monitor system. It is based on the development of the pulsatile unloading of the finger arterial walls using an inflatable finger cuff. During continuous BP measurement CO is calculated. We included 10 patients with standard invasive cardiac monitoring system (pulmonary artery catheter and arterial catheter) comparing invasively obtained data to the data collected noninvasively using the Nexfin HD.

Main results:

Correlation between mean arterial pressure measured with the standard arterial monitoring system and the Nexfin HD was r2 = 0.67 with a bias of -2 mmHg and two standard deviations of ± 16 mmHg. Correlation between CO derived from PAC and the Nexfin HD was r2 = 0.83 with a bias of 0.23 l/min and two standard deviations of ± 2.1 l/min; the percentage error was 29%.

Conclusion:

Although the noninvasive CO measurement appears promising, the noninvasive blood pressure assessment is clearly less reliable than the invasively measured blood pressure. Therefore, according to the present data application of the Nexfin HD monitoring system in the ICU cannot be recommended generally. Whether such a tool might be reliable in certain critically ill patients remains to be determined.

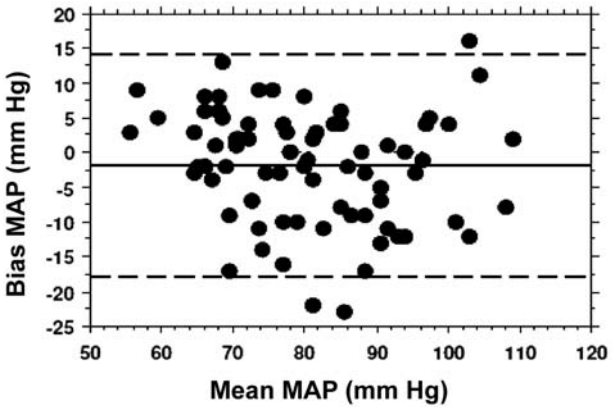

Figure 3
Bland Altman analysis comparing Nexfin HD with invasively measured arterial blood pressure.

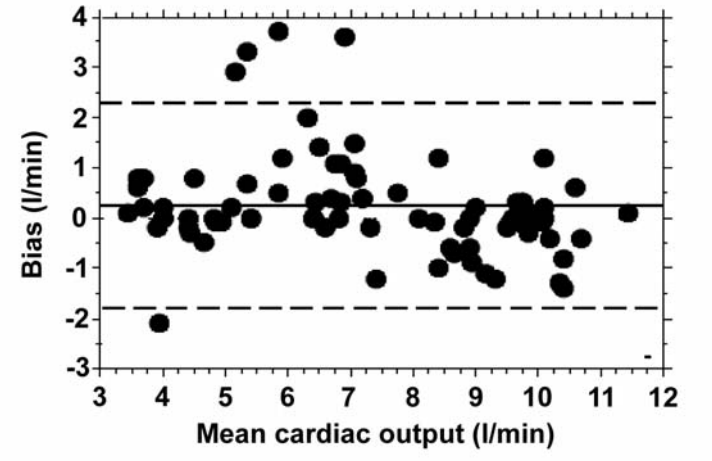

Figure 4
Bland Altman analysis comparing Nexfin HD with invasively measured cardiac output.

Collaborations:

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

Selected references:

- Flückiger C, Béchir M, Brenni M, Ludwig S, Sommerfeld J, Cottini SR, Keel M, Stocker R, Stover JF. Increasing hematocrit above 28% during early resuscitative phase is not associated with decreased mortality following severe traumatic brain injury. Acta Neurochir (Wien). 2009 Dec 24. [Epub ahead of print] PubMed PMID: 20033233.
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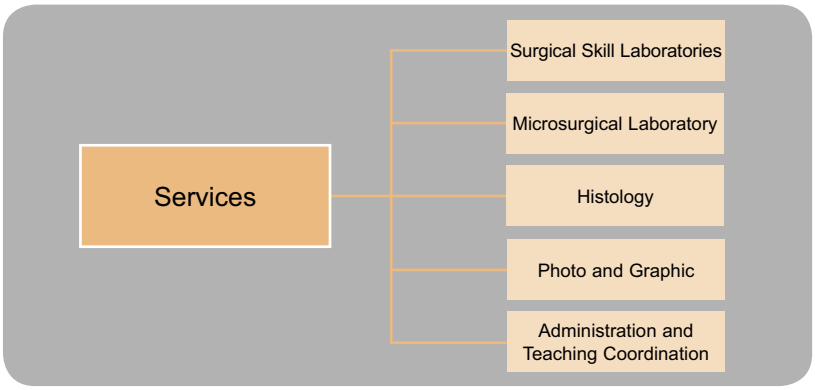
3. Services




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


Vlasta Strohmeier

3.2 Microsurgical Laboratory

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


Pia Fuchs

3.3 Histology

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

3.4 Photo and Graphic Services

Nico Wick,
Photographer

Lea Schütz-Cohen,
Photographer

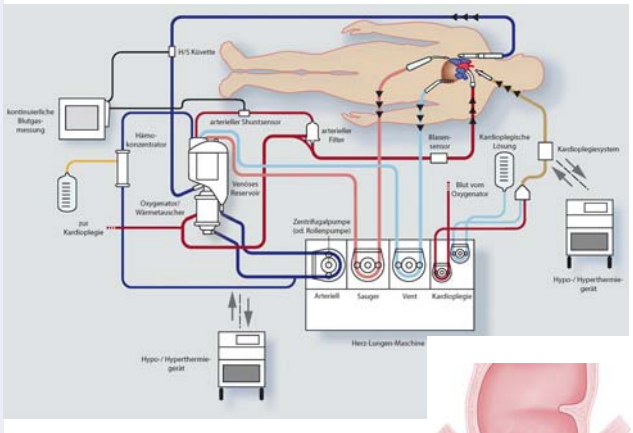
Stefan Schwyter,
Scientific
Illustrator

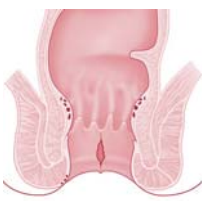
Carol De Simio,
Scientific
Illustrator

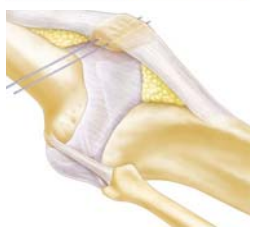
A quick, flexible, versatile and professional service.

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







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

Donata Gröflin
Teaching Coordinator
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.
- Coordination and organization of 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 2009

8th Day of Clinical Research

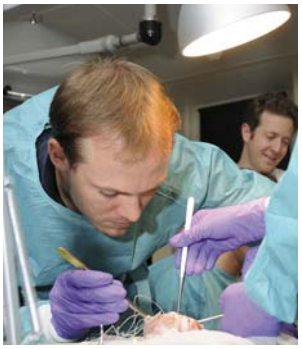
Injection class

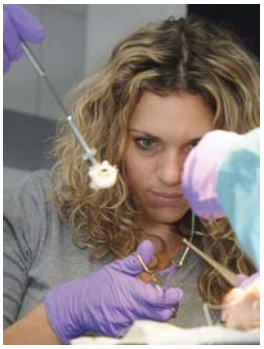
Inauguration small animal-MR, BZL

Goodbye Astrid Morger

Goodbye Dr. Li-Kang Sun

Aesculap sewing course for medical students





Wetlab course

Microsurgery course

Christmas party

Goodbye Vlasta Strohmeier

5. Publications 2009

- Aboushwareb, T; Eberli, D; Ward, C; Broda, C; Holcomb, J; Atala, A; Van Dyke, M (2009). A keratin biomaterial gel hemostat derived from human hair: evaluation in a rabbit model of lethal liver injury. In: Journal of Biomedical Materials Research. Part B: Applied Biomaterials 90(1), 45-54
- Altintas, A A; Altintas, M A; Ipaktchi, K; Guggenheim, M; Theodorou, P; Amini, P; Spilker, G (2009). Assessment of microcirculatory influence on cellular morphology in human burn wound healing using reflectance-mode-confocal microscopy. In: Wound Repair and Regeneration 17(4), 498-504
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6. Grants 2009

Cardiac Surgery

Grants	Title of Project	Project Leader
EU Grant Framework Program 6 (BioSys)	Intelligent Biomaterial Systems for Cardiovascular Tissue Repair	S.P. Hoerstrup
Swiss National Science Foundation	Development of Cell-Based Therapies for Myocardial Defects	S.P. Hoerstrup
Swiss Foundation for Research on Muscle Diseases	M-band alterations characterize muscle pathogenesis	I. Agarkova
Zurich Center for Integrative Human Physiology Grant 2006 – 2010	Vulnerable atherosclerotic plaques - early detection functional imaging, targeted treatment	S.P. Hoerstrup
Wolfermann-Nägeli-Foundation	The role of M-band in the striated muscle sarcomere	I. Agarkova
Roche Research Foundation	Sarcomere remodeling in the failing heart: implications for the disease mechanism	I. Agarkova
Fakultät der Universität Zürich	Sarcomeric M-band as a novel marker for the remodelling process in cardiomyopathy	R. Schönauer
Hartmann-Müller-Stiftung	Prenatal Human Progenitor Cells for Tissue Engineering of Living Autologous PediatricCardiovascular Replacements	D. Schmidt S.P. Hoerstrup
Novartis Stiftung	Tissue Engineering Heart Valves	G. Zünd S.P. Hoerstrup
National Competence Center of Research	Klinik für Herz- und Gefässchirurgie - Lead Cardiac Robotic Surgery Switzerland	J. Grünenfelder
CO-ME	Robotics in Cardiovascular surgery	G. Zünd J. Grünenfelder
Atricure inc.	Cosgrove Gillinov Clip Studie	S. Salzberg

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	P.A. Clavien
SNF	Hypothermic oxygenated perfusion extracorporeal of the rat liver in non heart beating donors after cold storage	P. Dutkowski
Sophienstiftung	Mechanism of human liver regeneration after major epatectomy and portal vein ligation using gene microarray technology	P.A. Clavien H. Petrowsky
Edoardo R., Giovanni, Giuseppe und Chiarina Sassella-Stiftung	Serotonin Antagonist	C. Soll P.A. Clavien
Roche Organ Transplantation Research	Protective Mechanisms of Pentoxyfilline for Liver Surgery and Liver Transplantation	H. Petrowsky P.A. Clavien
Désirée und Niels Yde Stiftung	Liver Cancer	J.H. Jang P.A. Clavien
Krebsliga	Pathways in HCC	C. Soll P.A. Clavien
Pancreatitis laboratory		
SNF	The role of COX-2 in chronic pancreatic inflammation and fibrosis	R. Graf
Gottfried und Julia Bangerter-Rhyner-Stiftung	Serotonin in Pancreas	R. Graf
Amelie Waring Stiftung	Chronische Pankreatitis	R. Graf
Amelie Waring Stiftung	Chromosal Aberrations and DNA Damage in Chronic Pancreatitis	P.A. Clavien M. Lesurtel

Plastic Hand & Reconstructive Surgery

Grants	Title of Project	Project Leader
Hartmann Müller-Stiftung	Tendon repair in hand surgery	J. Buschmann
Wolfermann-Nägeli-Stiftung	Tendon repair in hand surgery	J. Buschmann
Fonds für Medizinische Forschung - Universität Zürich	Tendon repair in hand surgery	J. Buschmann
Helmut Horten Stiftung	Role of exogenously administred recombinant erythropoietin in plastic surgery	C. Contaldo P. Giovanoli
Elite-med Stiftung Zürich	Microcirculation study	C. Contaldo

Thoracic Surgery

Grants	Title of Project	Project Leader
SNF	Immune targeted therapy for lung cancer	S. Hillinger
SNF	Trachea reconstruction using novel tissue engineered constructs	W. Weder
SNF	The Role of CD26/DPP IV and SDF-1 in pulmonary ischemic injury in a mouse lung transplantation model	W. Jungraithmayr
Krebsliga Zürich	Prognostic markers for malignant pleural mesothelioma	I. Schmitt-Opitz
Krebsliga Zürich	Establishment of an integrated tumor tissue platform and its application for comprehensive analyses of molecular parameters in lung tumors	W. Weder
Hartmann-Müller-Stiftung	The effect of NSAIDs on early inflammatory response after mechanical pleurodesis in a pig model	I. Schmitt-Opitz
Fellowship European Society of Medical Oncology	Intrapleural therapy after surgery for malignant pleural mesothelioma	I. Schmitt-Opitz
Lungen Liga 2009	Effect of N-Acetyl-cysteine on acute allograft rejection after lung transplantation	I.Inci
Lungen Liga 2009	Attenuation of ischemia-reperfusion injury by N-Acetylcysteine after lung transplantation	I.Inci
Lungen Liga 2009	Reconditioning of category 3 non-heart beating donor lungs insulted to gastric aspiration: Utilization of ex vivo lung perfusion system	I.Inci
Krebsliga	Epstein Barr virus-induced molecule 1 ligand chemokine in lung cancer therapy	S. Hillinger
Sassella-Stiftung	Epstein Barr virus-induced molecule 1 ligand chemokine (ELC/CCL19)	S. Hillinger
EMDO-Stiftung	Entwicklung eines in-vivo-Bioreaktors zur Reepithelialisierung einer tissue-engineerten Neo-Trachea	S. Hillinger
Deutsche Forschungsgemeinschaft	Entwicklung eines Modells der chronischen Abstossung	W. Jungraithmayr
Dr. U. Arnold and Susanne Huggenberger-Bischoff Stiftung zur Krebsforschung	Activity based protein profiling in human lung cancer biopsies	S. Arni
Krebsliga Zürich	Preclinical pharmacocinetic study for evaluation of intrapleural treatment with Cisplatin-fibrin after pneumonectomy for malignant pleural mesothelioma	I. Schmitt-Opitz
Becon AG	Prognostische Marker für das maligne Pleuramesothelioma	I. Schmitt-Opitz W. Weder
Matching Fund	Prognostic Marker for Malignant Pleural Mesothelioma	I. Schmitt-Opitz

Urological Research

Grants	Title of Project	Project Leader
SNF Projektförderung	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	M. Provenzano
SNF SCORE Förderung	Muscle Precursor Cells for the treatment of Urinary Incontinence	D. Eberli
Matching Fund des USZ	Muscle Precursor Cells for the treatment of Urinary Incontinence	D. Eberli
Eleonoren Stiftung	Innervation of Tissue Engineered Bladder Constructs for Functional Reconstruction	D. Eberli
Forschungskredit UZH	Characterization of CTL immune activitiy against p53-binding regions of BKV large T antigen in BKV seropositive prostate cancer patients	G. Sais M. Provenzano
Forschungskredit UZH Stiftung für med. Forschung	Adult Muscle Progenitor Cells for Clinical Applications: Function, Safety and Interactions	M. Stölting D. Eberli
Hartmann Müller Stiftung	Interaction between Adult Stem Cells (ASC) and pre-existing Cancer in Tissue Engineering	M. Stölting D. Eberli
EMDO Stiftung	Adult Muscle Progenitor Cells for Clinical Applications: Function, Safety and Interactions	M. Stölting D. Eberli

Cranio-Maxillofacial Surgery Research

Grants	Title of Project	Project Leader
SNF	Functional testing of diarthrodial joint soft tissues with in vivo acquired anatomical and kinematic information	L. Gallo F. E. Weber
SNF	Artificial mesenchymal progenitor cell niches for Bone tissue engineering	M. Lütolf F. E. Weber
SNF	Synthetic biomimetic hydrogels for dual delivery of growth factors and their enhancers	F. E. Weber
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	J. Vörös F. 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	J. Stover
Hartmann Müller Stiftung	Einfluss von Noradrenalin auf die Funktion isolierter arterieller und jugularvenöser Thrombo bei intensiv-pflichtigen Patienten mit schwerem SHT	J. Stover
Fresenius Kabi (Schweiz) AG	Early fluid resuscitation with balanced HES 130/0.4 [6\%] in severe burn injury	M. Béchir
UBS Wealth Management	Pathophysiologische Relevanz aktivierter Thrombo-zyten und Einfluss von Noradrenalin auf die Funktion isolierter Thrombozyten nach schwerem Schädel Hirn Trauma	J. Stover
Hartmann Müller Stiftung	Role of PAR1 in Vascular Barrier Regulation	R. Schüpbach
CSL Behring	Faktor XIII Substitution bei Patienten mit Naht- und Darm- Anastomoseninsuffizienzen - A randomized open interventional study	M. Béchir
SUVA Fonds	Improvement of therapy in patients with severe traumatic brain injury. differential impact of local and systemic changes and routinely applied drugs	J. Stover
Fresenius Kabi (Schweiz) AG	Pharmakokinetische und pharmakodynamische Charakterisierung der enteralen und parenteralen Glutamin (GLN) Supplementierung bei intensivpflichtigen Patienten mit schwerem Schädel Hirn Trauma	J. Stover

7. Awards 2009

- Dr. Daniel Eberli, MD, PhD: Prize of the Swiss Association of Urology for the research work performed in the field of urologic tissue engineering. Annual Meeting of the Schweizerischen Gesellschaft für Urologie, Lausanne, 2009
- Dr. med. Daniel Eberli et al.: Best Poster of the Session of the European Association of Urology. Annual meeting of the European Association of Urology, Stockholm, 2009
- Dr. med. Lukas J. Hefermehl et al.: Best Poster of the Session. Kongress der Deutschen Gesellschaft für Urologie, Düsseldorf , 2009
- Dr. Wolfgang Jungraithmayr: Prize of the Swiss Society for Thoracic Surgery for best publication 2009
- Dr. W. Jungraithmayr: best Presentation, Swiss Society for Thoracic Surgery, SGC Kongress Montreux
- Dr. med. Mickael Lesurtel, Götz-Preis der Universität Zürich
- PD Dr. med. Nicole Lindenblatt et al.: One of the Top 3 presentations at the 1st European Plastic Surgery Research Council 2009
- PD Dr. med. Nicole Lindenblatt et al.: Best research paper of the 45th Congress of the Swiss Society for Plastic, Reconstructive and Aesthetic Surgery 2009
- Dr. med. Alexander Müller et al.: Best Poster. 24th Annual EAU Congress, Stockholm, 2009
- Dr. med. Alexander Müller et al.: Best Poster. 61. Kongress der Deutschen Gesellschaft für Urologie e.V., Dresden, 2009
- Dr. med. Alexander Müller et al.: SGU-Poster Price. 65. Jahrestagung der Schweizerischen Gesellschaft für Urologie, Lausanne, 2009
- Krebsliga grant, ESMO Award
- Dr. Dörthe Schmidt: Pfizer Forschungspreis 2009 für Grundlagenforschung Herzkreislauf

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