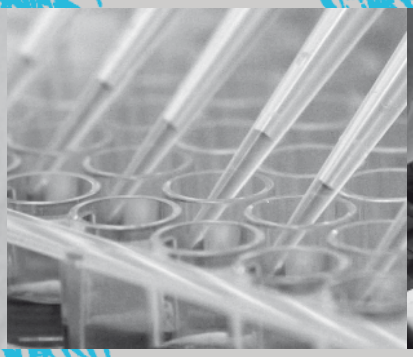
Division of Surgical Research

Annual Report 2005

Department of Surgery
University Hospital Zurich
Switzerland



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

Photo & Graphic, Division of Surgical Research
Nico Wick and Carol De Simio

Content

Preface

Dear Colleagues

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

It is my pleasure to present to you the Annual Report 2005 of the Division of Surgical Research, Department of Surgery at the University Hospital Zurich.

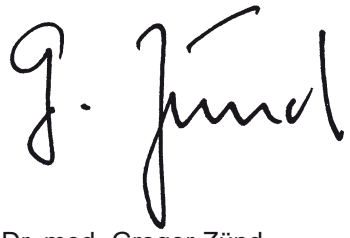
In the year 2005 we have undergone substantial administrative reorganisation and with the support of all members within the Division organisational problems could be solved quickly. The new facilities built in 2004 were successfully implemented in the daily routine and are highly appreciated by technicians and researchers. The research group of the Division of Surgical Intensive Care was integrated and could be provided with new laboratories.

The Division's quarterly internal reports comprising the corresponding operational expenditures as well as the investments carried out allow for a quality control by means of timely financial project management. The investments carried out in the past year included the acquisition of a high performance liquid chromatography (HPLC) and a reversal microscope.
Both acquisitions are being used by various research groups from our Division complementing the apparatus pool accordingly.

The weekly lectures held by the Division of Surgical Research at the University Hospital Zurich are regularly attended by the members of our Division and other researchers representing an integrative part of the academic curriculum within the University, the University Hospital and the Federal Institute of Technology

It is my pleasure to thank all members within our Division and research partners of the University, University Hospital and Federal Institute of Zurich for the excellent performance and collaboration last year.

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.
Michele Genoni
Director Clinic
Cardiac Surgery

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

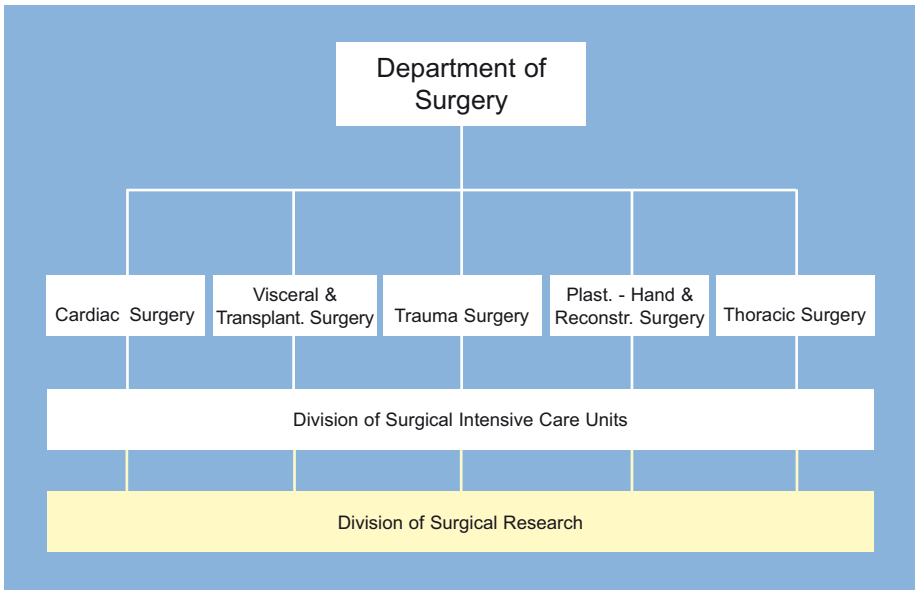
Prof. Dr. med.
Otmar Trentz,
Director Clinic
Trauma Surgery

Dr. med.
Walter Kuenzi,
Director a.i. Clinic
Plast. - Hand &
Reconstr. Surgery

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

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

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

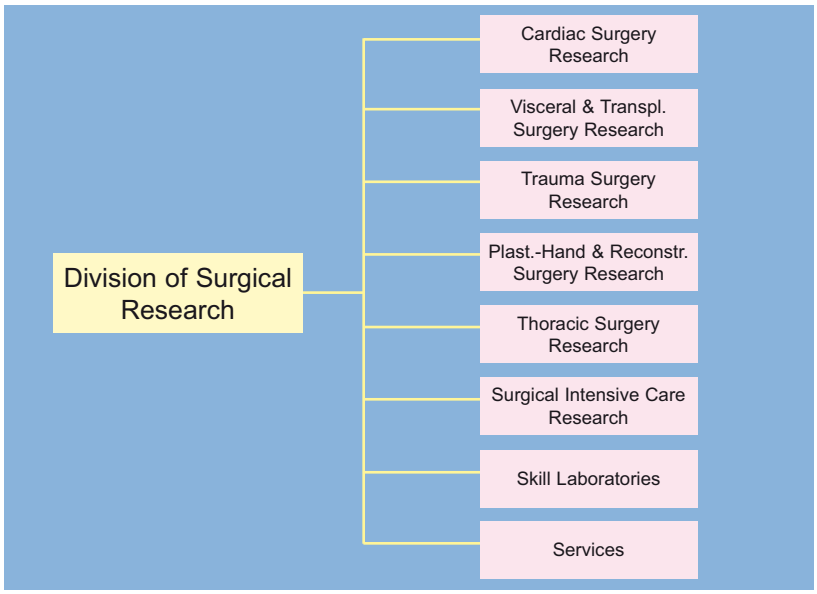


1.2 Structural Organisation of the Division of Surgical Research

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

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

Susanne Frehner,
Administration
Division of Surgical
Research



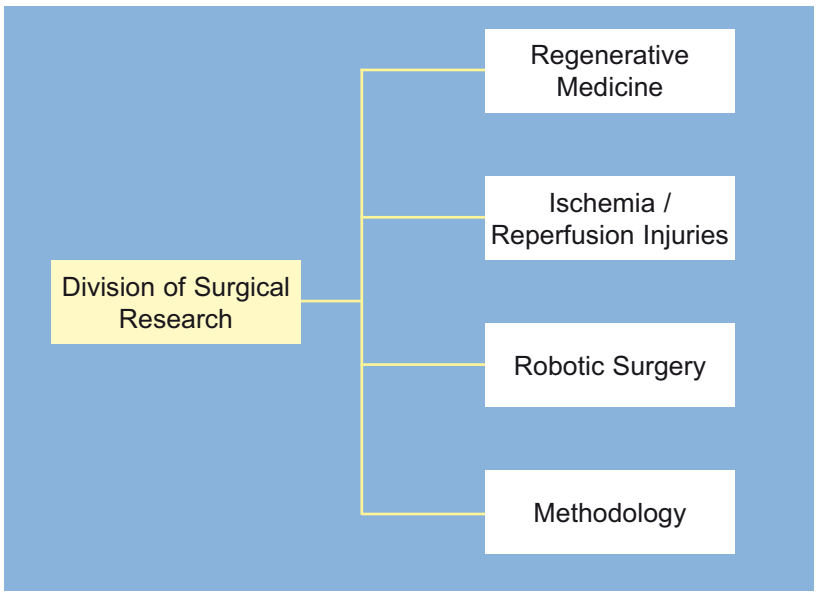
1.3 Scientific Sections within the Division of Surgical Research

Prof. Dr. med.
Simon Philipp
Hoerstrup
Regenerative
Medicine

Dr. phil II
Wolfgang Moritz
Ischemia /
Reperfusion
Injuries

PD Dr. med.
Guido Wanner,
Robotic Surgery

PD Dr. phil II
Rolf Graf
Methodology



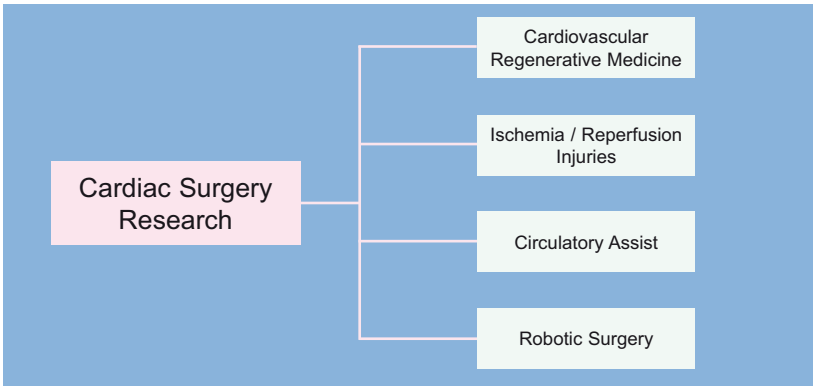
2. Research and Development

2.1 Cardiac Surgery Research


Prof. Dr. med. Simon Philipp Hoerstrup


Prof. Dr. med. Gregor Zünd


Prof. Dr. med. Michele Genoni



2.1.1 Cardiovascular Regenerative Medicine (Tissue Engineering and Cell Transplantation)

Prof. Dr. med. Simon Philipp Hoerstrup
(Director, Regenerative Medicine Program; Professor of Biomedical Engineering TU/e)


Prof. Dr. med. Simon Philipp Hoerstrup


Dr. sc. nat. Jens Kelm


Dr. med. Dörthe Schmidt


PD Dr. sc. nat. Stefan Neuenschwander


Dr. med. Ian Cummings


Anita Mol PhD Student


Dr. med. Alberto Weber


Sirpa Price


Prof. Dr. Vijay Kumar


cand. med. Armin Zürcher


cand. med. Silvan Holdener

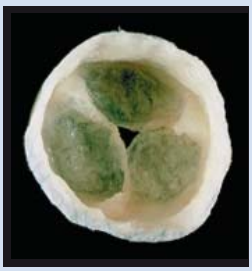

cand. med. Sandro Imbach

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.

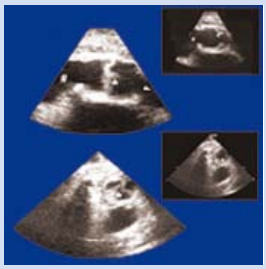
Research projects:

- Human Cell-Based Systems (progenitor, foetal, adult)
- Extracellular Matrix (proteins, tensegrity)
- Biomaterials (biodegradable, bio-active)
- Bioreactor Systems (tension, flow)
- 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

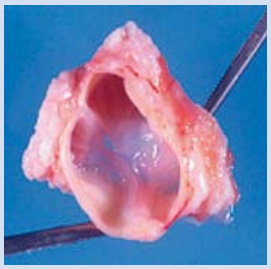
Proof of Heart Valve Tissue Engineering Concept



pre-implantation



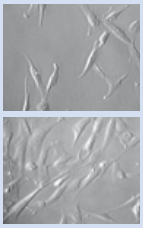
in-vivo

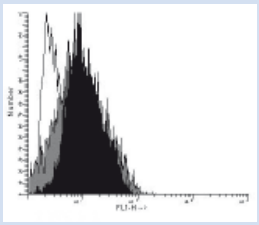


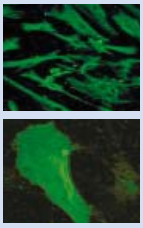
post-explantation

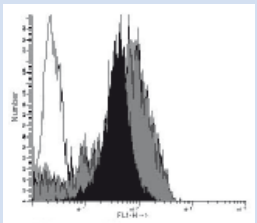
Autologous living tissue engineered heart valve in a sheep model, based on vascular - derived myofibroblasts and endothelial cells *(Hoerstrup et al, Circulation 2000)*

Human Heart Valve Tissue Engineering





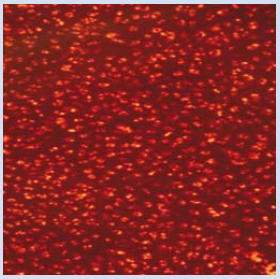




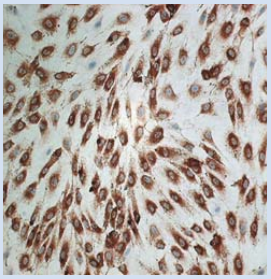
Heart valve tissue engineered from human marrow stromal cells
(Hoerstrup et al. Circulation 2002)

Human Prenatal Progenitor Cells

Native EPCs

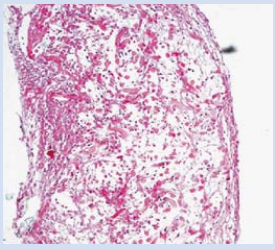


Uptake of ac-LDL

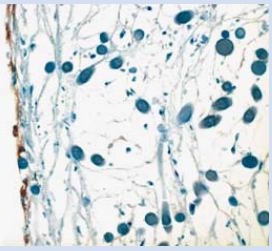


vWF staining

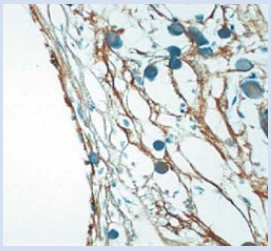
Human endothelial progenitor cells isolated from human umbilical cord blood
Schmidt et al. Ann Thorac Surg. 2004



H&E staining



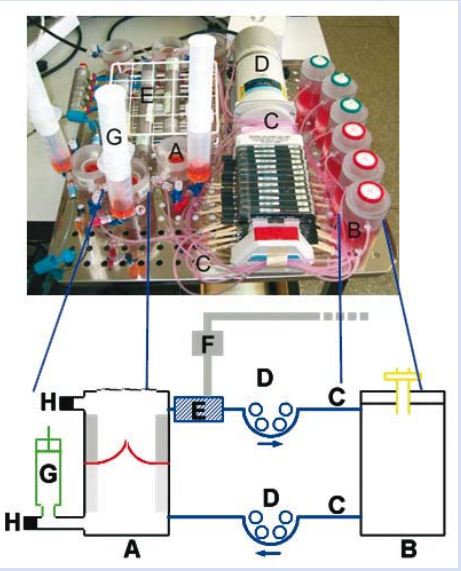
CD 31staining



vWF staining

Human umbilical cord-derived endothelial progenitor cells seeded onto a living matrix engineered from umbilical cord tissue
Schmidt et al. EJCTS 2005

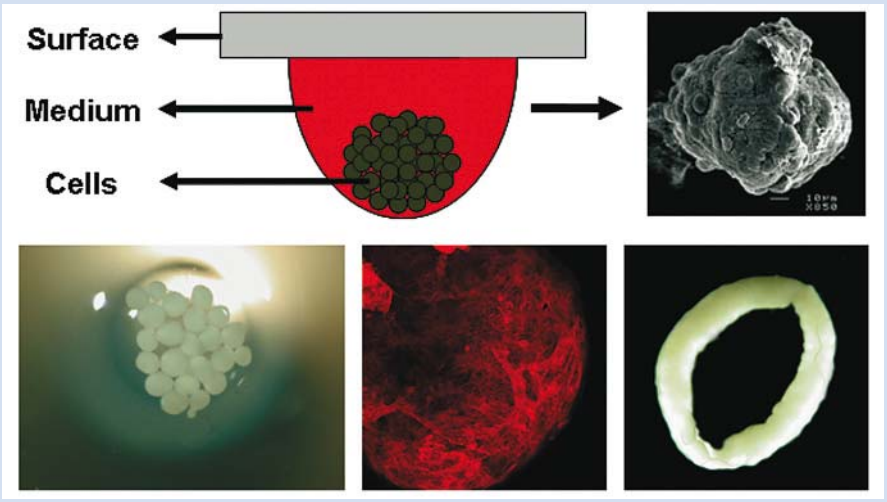
Bioreactor Development



- sized small
- biocompatible materials
- maintenance of sterility
- static conditioning by continuous perfusion
- dynamic conditioning by mimicking diastolic phase

Mol et al. Ann Biomed Eng 2005

Microscale TE for myocardial Regeneration



Gravity-mediated production of myocardial microtissue grafts. Myocardial microtissues retain heart-muscle specific morphology (as shown by fluorescent staining of sarcomeric alpha-actinin), which can be used as cell grafts or as building blocks to assemble larger tissue patches of any desired shape.

Achievements 2005

- Fed. Commission for Technology and Innovation Grant (CTI) Hoerstrup SP
- Swiss National Foundation (NFP 46), Hoerstrup SP
- EU Grant Framework Program 6 (Biosys), Horstrup SP
- Bundesministerium für Bildung und Forschung (BMBF Grant), Hoerstrup SP
- Hartmann-Müller-Stiftung, Schmidt D
- Best Oral Presentation 4th Day of Clinical Research 2005, ZKF, Zürich, Switzerland, Schmidt D

Collaborations

- Department of Biomedical Engineering, Technical University Eindhoven, The Netherlands
- Center for Integrative Human Physiology, University of Zurich, Switzerland
- Department of Materials, Federal Institute of Technology, Zürich,Switzerland
- Department of Biochemistry, University Zürich, Switzerland
- Department of Mathematics, Federal Institute of Technology, Zürich, Switzerland
- Department of Computational Science, Federal Institute of Technology, Zürich, Switzerland
- Department of Veterinary Surgery, MSRU Vetclinics, University Zürich, Switzerland
- Department of Cardiology, University Hospital Zürich, Switzerland
- Department of Cardiac Surgery, Children's Hospital, Harvard Medical School, Boston, MA, USA
- Department of Pathology, Brigham and Women‘s Hospital, Harvard Medical School, Boston, MA, USA
- Massachusetts Institute of Technology (MIT), Cambridge, MA, USA
- Laboratory for Tissue Engineering, German Heart Centre, Berlin, Germany
- Laboratory for Transplantation Immunology, University Hospital Zürich, Switzerland
- Institute of Chemistry and Applied Biosciences, Federal Institute of Technology Zürich, Switzerland
- Institute of Anatomie, University of Bern, Switzerland
- Feto-maternal Hematology Research, Department of Obstetrics, University Hospital Zürich, Switzerland

Selected references:

- Hoerstrup SP, Sodian R, Daebritz S, Wang J, Bacha E A, Martin D P, Moran A M, Guleserian K J, Sperling J S, Hatsuoka S, Kaushal S, Vacanti J P, Schoen F J, Mayer J E (2000) Functional living trileaflet heart valves grown in vitro. Circulation 102(III): 44-49
- Hoerstrup SP, Kadner A, Melnitchouk S, Trojan A, Eid K, Tracy J, Sodian R, Visjager J, Kolb S, Grunenfelder J, Zund G, Turina M (2002) Tissue engineering of functional trileaflet heart valves from human marrow stromal cells. Circulation 106: I-143-150
- Schmidt D, Mol A, Neuschwander S, Breymann C, Gossi M, Zund G, Turina M, Hoerstrup SP. Living patches engineered from human umbical cord derived fibroblasts and endothelial progenitor cells. Eur J Cardiothorac Surg. 2005 May; 27(5):795-800.
- Mol A, Driessen NJ, Rutten MC, Hoerstrup SP, Bouten, CV, Baaijens FP. Tissue engineering of human heart valve leaflets: a novel bioreactor for a strain-based conditioning approach. Ann Biomed Eng. 2005 Dec;33(12):1778-88
- Kelm JM, Djonov V, Hoerstrup SP, Guenter CI, Ittner LM, Greve F, Hierlemann A, Diaz Sanchez-Bustamante C, Perriard JC, Ehler E and Fussenegger M. Tissue-Transplant Fusion and Vascularization of Myocardial Micro- and Macrotissues Implanted into Chicken Embryos and Rats. Tissue Eng. 2006 in press.

2.1.2 Ischemia / Reperfusion Injury

PD Dr. med.
Reza Tavakoli

Dr. rer. nat.
Anna Bogdanova

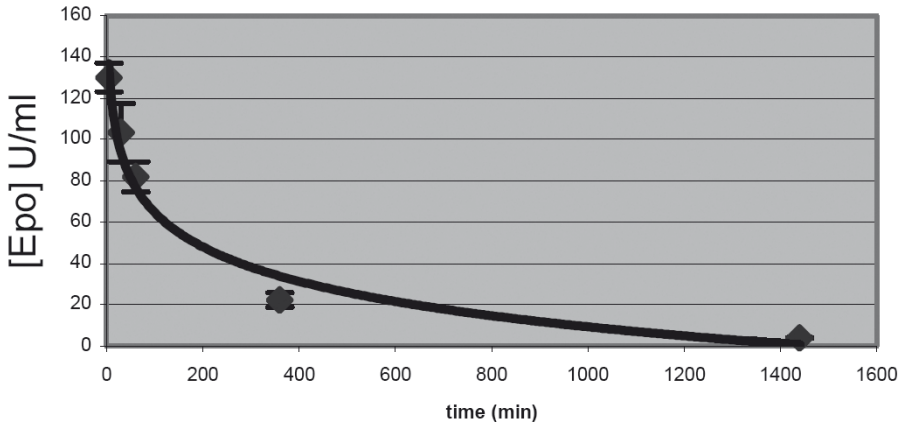
Deyan Mihof
PhD

Antiapoptotic effect of Erythropoietin and Lithium in myocardial reperfusion injury

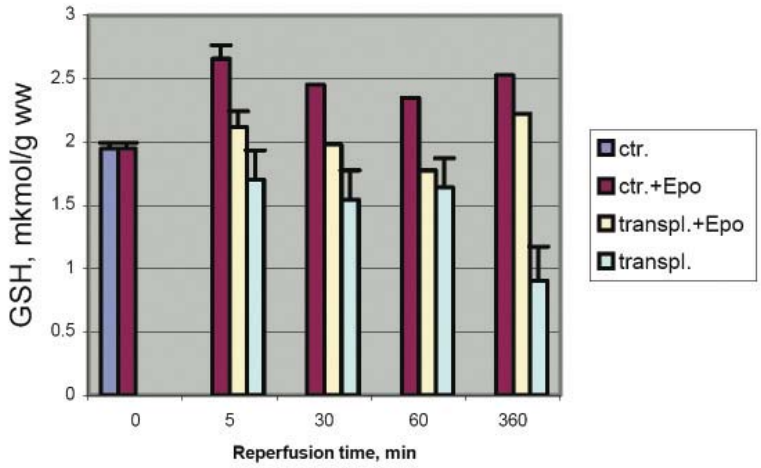
PD Dr. med. R. Tavakoli, Dr. rer.nat. A. Bogdanova, Dr. D. Mihof, Dr. A. Weber, V. Strohmeier

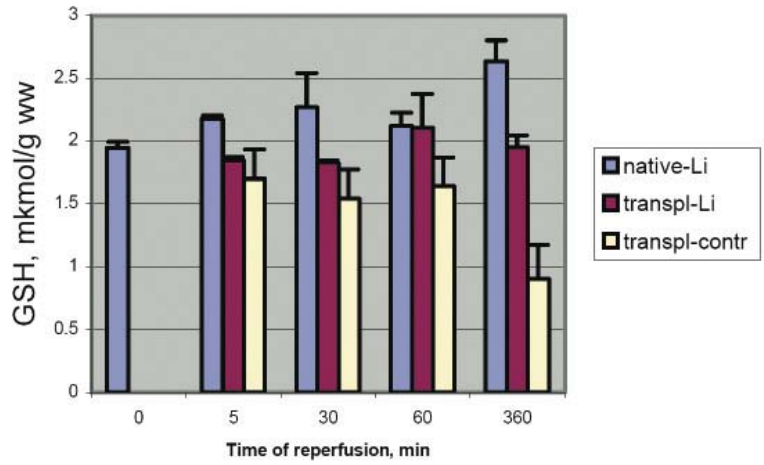
In our previous study the protective effect of antioxidant therapy was investigated in a heterotopic heart transplant model in the rat. In the present study we investigate the cardioprotective potential of erythropoietin and lithium salts against ischemia-reperfusion injury.
Heterotopic heart transplantation is performed from male Lewis donor Rats (250-300 g) to male Lewis recipient Rats (250-300 g). Recipient rats are divided in 3 groups: -Group Epo receives 5000U/Kg Eprex (human recombinant Erythropoietin) 20 min. prior to reperfusion –Group Li receives 10mg/Kg LiCl 20 min.prior to reperfusion –Group C receives no treatment.
In each group recipients are sacrificed at 5 time points: 5, 30, 60, 360 and 1440 min. after reperfusion. Redox status is measured by tissue Gluthation content (GSH), myocardial injury by Troponin T and water content. Apoptosis is investigated by measurements of GSK, Akt-cascade, caspase 7.
Twenty four hours after the operation Epo levels in plasma are still exceeding the basal level 49-fold (3 U/ml vs 61±6 mU/ml). Lithium treatment does not cause any changes in plasma Epo levels.

Epo-plasma levels in rats treated with rHuEpo

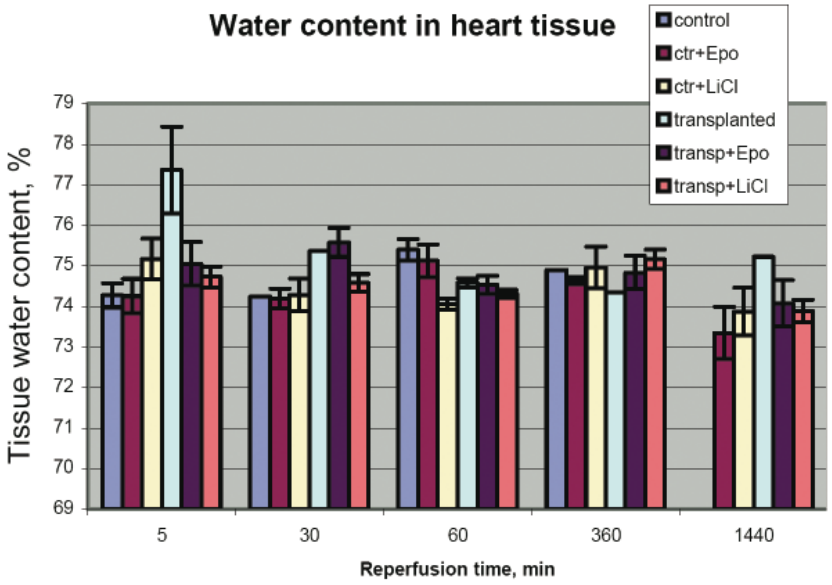


Transplanted hearts treated by Epo show no GSH deprivation in contrast to the grafts of non-treated control animals. Interestingly, Li^+ is as efficient as Epo in preventing reperfusion-induced GSH depletion (Fig 2B). In contrast to the action at the heart tissue level, Li^+ treatment causes a decrease in GSH content of erythrocytes of the recipient animals. Mechanisms of GSH regulation by lithium are thus tissue-specific. Epo treatment is without an effect on red cell GSH content.





In agreement with our previous findings, swelling of the graft tissue of the control group 5 min after the onset of reperfusion is due to the Na^+ accumulation. Administration of Epo or Li^+ abolishes reperfusion-induced swelling and Na^+ accumulation.



Both Epo and Li+ stimulate GSH production in myocardial tissue and thus protect the heart from the oxidative stress induced by reperfusion.
Furthermore, stress and tissue damage markers (TnT, ANP, BNP) will be measured in plasma and finally, activity of the members of Akt-cascade will be monitored and apoptosis intensity in the grafts will be evaluated.

Achievement 2005

- Tavakoli R, Bogdanova A, Ossent P, Grenacher B, Bogdanova N, Gassmann M, Zund G, Genoni M. Multiparametric characterization of myocardial ischemia-reperfusion injury. Submitted for publication.

Collaborations:

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

Selected references:

- Wright, G.L. et al. Erythropoietin receptor expression in adult rat cardiomyocytes is associated with an acute cardioprotective effect for recombinant erythropoietin during ischemia-reperfusion injury. Faseb J 18, 1031-3 (2004).
- Parsa, C.J. et al. Cardioprotective effects of erythropoietin in the reperfused ischemic heart: a potential role for cardiac fibroblasts. J Biol Chem 279, 20655-62 (2004).
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- Ananthakrishnan, R., Hallam, K., Li, Q. & Ramasamy, R. JAK-STAT pathway in cardiac ischemic stress. Vascul Pharmacol 43, 353-6 (2005).
- Chalecka-Franaszek, E. & Chuang, D.M. Lithium activates the serine/threonine kinase Akt-1 and suppresses glutamate-induced inhibition of Akt-1 activity in neurons. Proc.Natl.Acad.Sci.U.S.A 20;96, 8745-8750 (1999).
- Jope, R.S. Lithium and GSK-3: one inhibitor, two inhibitory actions, multiple outcomes. Trends Pharmacol Sci 24, 441-3 (2003).
- Tramontano, A.F. et al. Erythropoietin protects cardiac myocytes from hypoxia-induced apoptosis through an Akt-dependent pathway. Biochem Biophys Res Commun 308, 990-4 (2003).

2.1.3 Mechanical circulatory support

PD Dr. med.
Mario Lachat

Prof. Dr. med.
Rene Pretre

PD Dr. med.
Markus Wilhelm

Dr. med.
Hitendu Dave

PD Dr. Mario Lachat, Prof. René Prêtre, PD Dr. Markus Wilhelm,

Long-term support

In 2005, the mechanical circulatory support program expanded further. Seven patients with terminal heart failure, who were in severe low-output syndrome refractory to medical therapy, were supported with the Berlin Heart INCOR, a magnetically suspended and intracorporeally implanted axial-flow pump for left ventricular support, which was introduced in our clinic in November 2004 (fig. 1). The success with this pump was extraordinary. Four patients were transplanted successfully, three patients are presently still on support awaiting transplantation. The longest support time is currently 6 months. Complication rate is very low, and quality of life is excellent. Five patients could be discharged home, and two patients went back to work while being on support and waiting for heart transplantation.
The Berlin Heart EXCOR is an extracorporeally located pulsatile pump (fig. 2). It can be used for biventricular or right ventricular support for which the INCOR is not the appropriate device. One patient was supported with an EXCOR right ventricular pump for right ventricular failure following orthotopic heart transplantation. After five months, the device could be weaned and explanted, and the patient is currently awaiting hospital discharge.

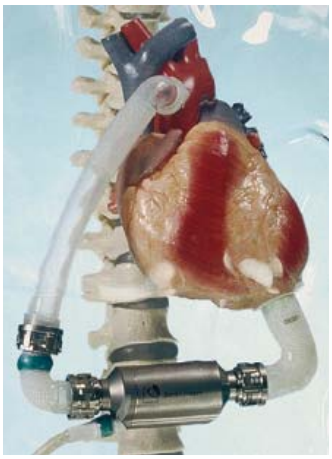


Figure 1 Berlin Heart INCOR

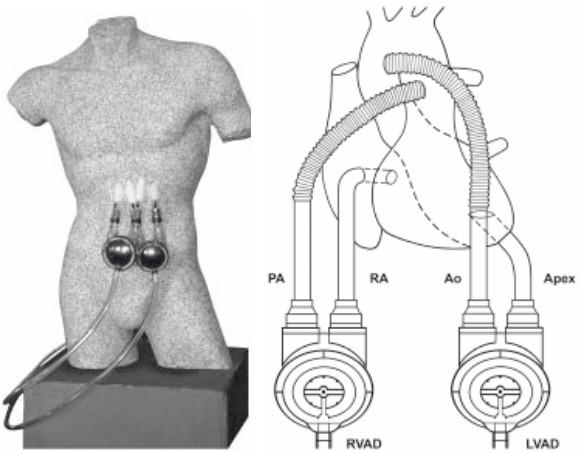
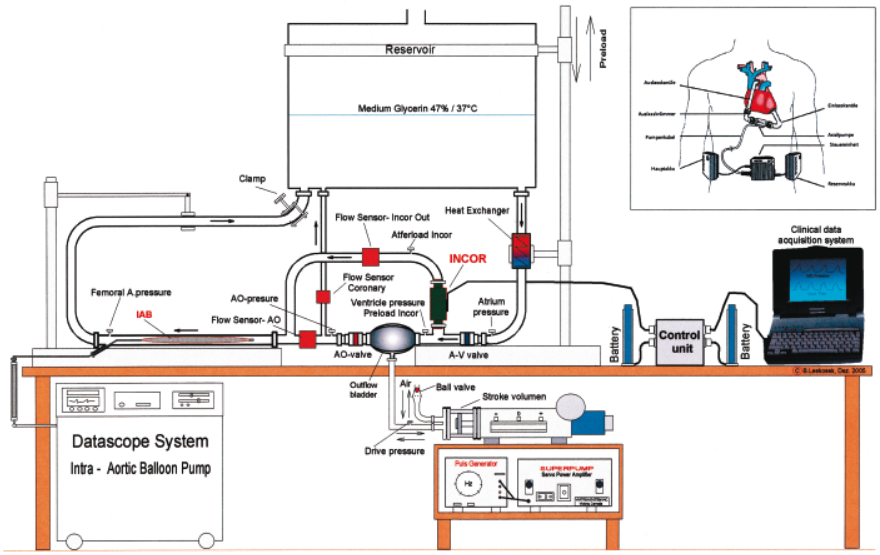


Figure 2 Berlin Heart EXCOR (links: extrakorporale Lage, rechts: Implantationsprinzip)

Short-term support

In 2005, ten patients were supported with ECMO (extracorporeal membrane oxygenation) for acute heart and lung failure, respectively. 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 ECMO was implanted in patients with ARDS due to causes such as fulminant pneumonia. ECMO support extended up to 3 weeks with good mechanical reliability.

INCOR (Berlin Heart) - Performance in vitro with IABP



Achievements 2005

- Mechanical circulatory support program with excellent bridge-to-transplant rate

Collaborations:

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

Selected references:

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2.1.4 Robotic Surgery and Innovative Technologies

PD Dr. med.
Jürg Grünenfelder

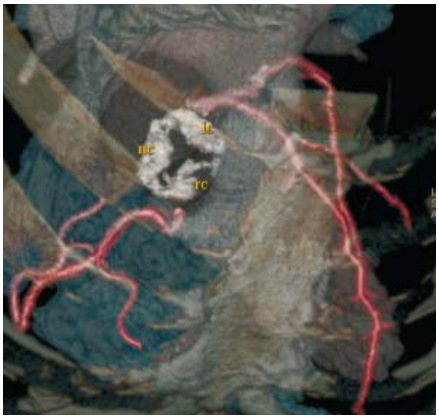
Dr. med.
André Plass

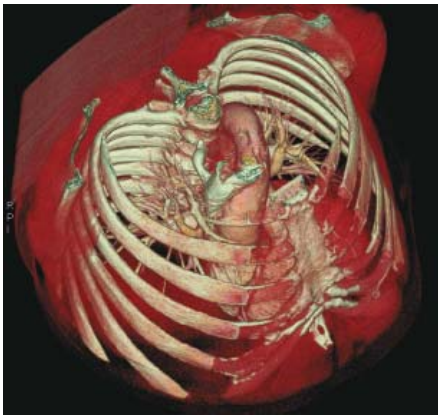
Minimally invasive cardiac surgery

PD Dr. med. Jürg Grünenfelder

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

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





Achievements 2005

- Swiss national scientific foundation grant for computer aided and image guided medical intervention (CO-ME)

Collaborations:

- Departement of Radiology, Universtiy Hospital Zürich (Hatem Alkadhi, MD)
- Laboratory for Thermodynamics in Emerging Technologies, ETH Zürich (Prof. Dimos Poulikakos)
- Institute of Mechatronic Systems, ZHW (Prof. Charles Brom)
- Physical Electronics Laboratory, ETH Zürich (Prof. Henry Baltes)

Selected references:

- Dynamic cine imaging of the mitral valve with 16-MDCT: a feasibility study.
  Alkadhi H, Bettex D, Wildermuth S, Baumert B, Plass A, Grunenfelder J, Desbiolles L, Marincek B, Boehm T.
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- Accuracy of MSCT coronary angiography with 64-slice technology: first experience.
  Leschka S, Alkadhi H, Plass A, Desbiolles L, Grunenfelder J, Marincek B, Wildermuth S.
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- Off-pump coronary artery bypass grafting: the Zurich experience.
  Tavakoli R, Reuthebuch O, Hofer C, Grunenfelder J, Genoni M.
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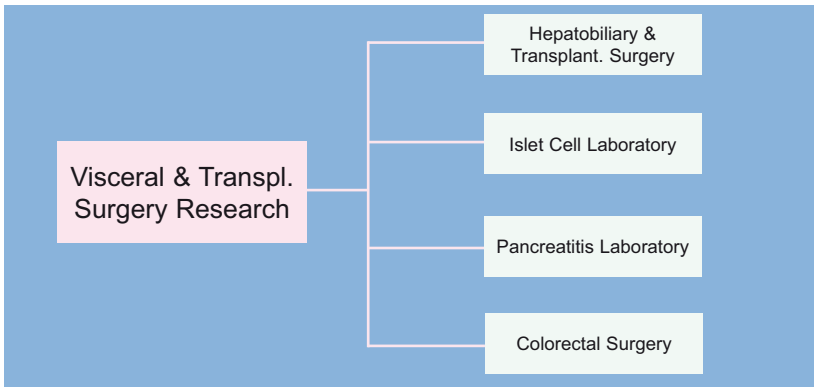
2.2 Visceral & Transplant Surgery Research

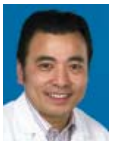
PD Dr. phil II
Rolf Graf

Prof. Dr. med.
Alain Clavien



2.2.1 Hepatobiliary & Transplantation Surgery

Clin. Ass. Prof.
Dr. med. Markus
Selzner

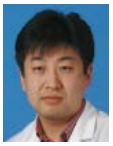


PD Dr. med.
Yinghua Tian

Dr. med.
Panco Georgiev

Dr. med.
Harm Hoekstra

Dr. des.
Ashraf Osman



Dr. nat.
Jae-Hwi Jang PhD

Dr. med.
Antonio Nocito

Udo Ungethüm

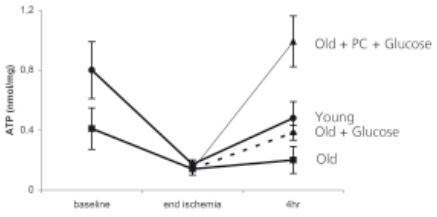
Ischemia / Reperfusion Injury and Liver Transplantation
PD Dr. med. Yinghua Tian; PD Dr. med. Markus Selzner, Dr. med. Nazia Selzner, Dr. med. Harm Hoekstra, Dr. med. Panco Georgiev, Dr. J.H. Jang, Dr. des. Ashraf Osman, Dr. med. Antonio Nocito

Ischemia/reperfusion injury of the liver represents an important problem in major hepatic surgery, liver transplantation, shock and trauma. Two types of injury have to be differentiated: (1) warm ischemic injury, occurring during liver resection, trauma and shock and (2) cold ischemic injury, occurring during organ preservation. Our group has a longstanding interest in the cellular processes leading to these two types of injuries. Additional factors affecting the outcome after ischemia and reperfusion are the presence of steatosis, cholestasis, cirrhosis and the age of the liver.
To assess ischemic injury in the fatty liver, intravital microscopy was used. We could show that the type of steatosis (macro- versus microsteatosis) has a strong impact on various parameters of liver perfusion and activity of leukocytes. Protective strategies e.g. ischemic preconditioning and intermittent clamping could improve these parameters. These results are in accordance with a recent publication in which the impact of steatosis on liver injury after ischemia and reperfusion was demonstrated (Selzner *et al* 2006).
Currently, investigations into the ability of the old liver to tolerate ischemic injury are performed. Results indicate that old livers are more prone to tissue injury which can be partially corrected by supplying glucose prior to a surgical insult. Here we could show that the ATP content of the liver was dramatically increased after a supplement of glucose. Concurrently, the injury was strongly reduced.

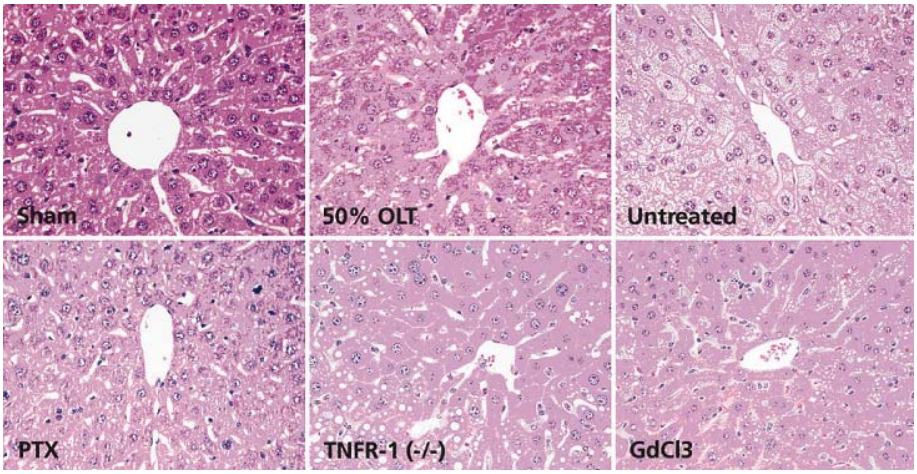
Against the background of platelets mediating sinusoidal endothelial cell apoptosis in cold hepatic ischemia, we investigated in a further project the role of platelets in normothermic ischemia and reperfusion injury.
We therefore used a model of platelet dysfunction and immune thrombocytopenia. Platelet aggregation in mice was inhibited by Clopidogrel feeding.
Immune thrombocytopenia was induced by intraperitoneal injection of anti-CD41 antibody. Subsequently, all mice were subjected to sixty minutes of partial hepatic ischemia and various time points of reperfusion.

Hepatic injury was determined by measurement of transaminase levels and by histological analysis of necrotic area and leukocyte infiltration. In addition liver repair after ischemic injury was investigated in platelet depleted animals and in mice lacking peripheral serotonin, since we have recently shown that platelet derived serotonin mediates liver regeneration.
In this study we could show that neither inhibition of platelet aggregation nor platelet depletion led to an improvement of I/R injury. However, liver regeneration and remodelling were significantly impaired in platelet depleted animals. Conversely, mice lacking peripheral serotonin were only deficient in liver regeneration.
From these results we concluded that platelets have no direct impact on the pathogenesis of normothermic I/R-injury but mediate tissue remodelling and liver regeneration. In addition, we demonstrate that platelet derived serotonin is also a specific mediator of regeneration in the postischemic liver.

Intra-hepatic ATP contents in young (●) and old (■) mice subjected to ischemic injury alone, in old mice with D-glucose injection prior to ischemia (◆), and old mice receiving D-glucose injection plus ischemic preconditioning prior to ischemic injury (▲). Administration of D-glucose resulted in a significant increase in intrahepatic ATP levels after ischemia/ reperfusion in old mice. Combining preconditioning and glucose administration resulted in an even more dramatic increase in intrahepatic ATP contents.



The mouse orthotopic liver transplantation (OLT) model was further developed: it was shown that 30% transplantation of a mouse liver results in a small-for-size syndrome with high morbidity and mortality. Various approaches to protect the small-for-size liver syndrome resulted in the detection of a mechanism involving TNF-α and Kupffer cells. Indeed, application of pentoxifylline, a drug routinely used in clinical therapy for thrombosis, was successful in reverting the small-for-size syndrome. Pentoxifylline suppresses TNF-α synthesis and has rheological activities, improving blood circulation (Tian et al 2006 PNAS, *in press*).



Histological analysis of graft tissue after transplantation.
H&E staining of partial OLT grafts was performed 2 days after transplantation. In contrast to untreated 30% partial OLT grafts, which revealed diffuse microvesicular steatosis, only mild macrovesicular steatosis was found in 30% partial OLT grafts after treatment with pentoxifylline and $GdCl_3$ (impairs Kupffer cell function) and in TNFR-1(-/-)grafts (disrupts the TNFα signalling pathway).

The effect of toxic bile on cold reperfusion injury was assessed. In a model of phospholipid depletion (Mdr2 knockout mice) we could show that altered bile composition affects the pathology of the bile duct and concurrently leads to increased liver injury after transplantation (Hoekstra et al Hepatology 2006, *in press*).

Many patients are affected by cholestasis prior to surgery. Hence the question was raised whether this condition has an impact on surgical interventions. A model of total bile duct obstruction was established. Mice used in these experiments exhibited strongly increased levels of bilirubin, indicators of cholestasis. We could show that cholestatic mice were protected from ischemic injury. The mediator of the protective effect is systemic which could be experimentally shown by selective obstruction of individual liver lobes.
In further experiments, the development of cholestasis in a complete bile-duct ligation model was established. Markers of injury and bile-duct proliferation were established and quantified. This long-term experiment will provide the basis for subsequent studies into the mechanisms of injury during cholestasis.
An additional project is currently focusing on the cirrhotic liver. We are trying to understand whether surgical and pharmacological strategies can protect the cirrhotic liver from ischemic injury. A model of liver cirrhosis was used that exhibits extensive tissue rearrangement (CCl_4).

Achievements 2005

Scientific

- We used a unique model of arterialized partial liver transplantation in mice. We could show that recipients of small-for-size livers (30%) have an improved survival rate after treatment with pentoxifylline.
- Establishment of mouse models of cholestasis
- The role of energy charge in aging livers: pre-treatment of animals with glucose reduces ischemic injury of the liver.
- Investigations into the mechanism of ischemia reperfusion injury in fatty livers led to the conclusion that these livers exhibited reduced sinusoidal perfusion and impaired leukocyte function.
- Grant awarded by the SNF.

Personnel

PD Dr. Tian received a poster prize from the ZKF (4th Day of Clinical Research)

Collaborations:

- PD Dr. W. Jochum, Institut für Klinische Pathologie, UniversitätsSpital Zürich
- PD Dr. B. Ludwig , Forschungsabteilung, Kantonsspital St.Gallen

Selected references:

- N Selzner, HA Rüdiger, R Graf, PA Clavien. Protective strategies against ischemic injury of the liver. Gastroenterology 2003;125:917-936.
- Dahm F, Georgiev P, Clavien PA. Small-for-Size Syndrome After Partial Liver Transplantation: Definition, Mechanisms of Disease and Clinical Implications. Am J Transpl 2005;5(11):2605-2610.
- N. Selzner, M. Selzner, W. Jochum, R. Graf, B. Ammann-Vesti, P.-A. Clavien. Mouse Livers with Macrosteatosis are more Susceptible to Normothermic Ischemic Injury than those with Microsteatosis. J. Hepatology 2005, *in press*.
- Y. Tian, W. Jochum, P. Georgiev, W. Moritz, Rolf Graf, and P.A. Clavien. Kupffer Cell dependent TNF-α Signaling mediates injury in the Arterialized Small-for-Size Liver Transplantation in the Mouse. PNAS 2006, *in press*

Dr. med.
Nazia Malekkiani
Selzner

Dr. med.
Mickael Lesurtel

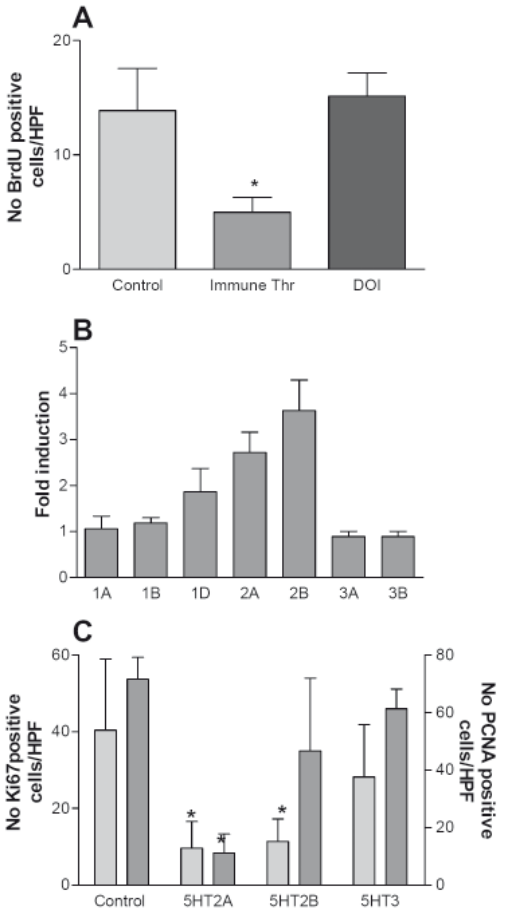
Dr. med.
Stefan Heinrich

Dr. med.
Katarzyna Furrer

Liver regeneration

Dr. med. Nazia Selzner, Dr. med. Mickael Lesurtel, Dr. med. Stefan Heinrich
Dr. med. K. Furrer

The liver is the only solid organ with the capacity to regenerate its volume after major tissue loss. Previous studies have shown that platelets are involved in a critical step during regeneration. To identify mediators of hepatocyte proliferation, soluble factors generated by platelets were investigated. One factor with the capacity to induce proliferation, serotonin, was analyzed. Animal studies with serotonin agonists were used to prove that serotonin may be a key mediator. Thrombocytopenic animals which do not regenerate their liver after 70% liver resection, received the serotonin agonist. This could reconstitute hepatocyte proliferation. Further studies, including knock-out mice for an enzyme involved in serotonin biosynthesis, could show that serotonin was a decisive factor in the induction of hepatocyte proliferation.



Demonstration of reduced liver regeneration in partially hepatectomized mice after depletion of platelets i.e. after immune thrombocytopenia (**A**).
Hepatocyte proliferation could be recovered by injection of a serotoinin agonist (DOI).
B: Relative regulation of transcripts coding for serotonin receptor subtypes after partial hepatectomy.
C: Mice treated with serotonin antagonists prior to and after resection. The 5HT2A receptor antagonist completely blocked regeneration, supporting the conclusion that serotonin is critically involved in liver regeneration.

Achievements 2005

Scientific

- Demonstration that serotonin is a key mediator in liver regeneration

Collaborations:

- PD Dr. Odermatt, Labor für Molekulare Diagnostik, Institut für Klinische Pathologie
- PD Dr. W. Jochum, Institut für Klinische Pathologie
- Prof. M. Bader, Max Delbrück Center for Molecular Medicine, Berlin

Selected references:

- Selzner N, Selzner M, Odermatt B, Tian Y, Van Rooijen N, Clavien PA. ICAM-1 triggers liver regeneration through leukocyte recruitment and Kupffer cell-dependent release of TNF-alpha/IL-6 in mice. Gastroenterology. 2003 Mar;124:692-700.

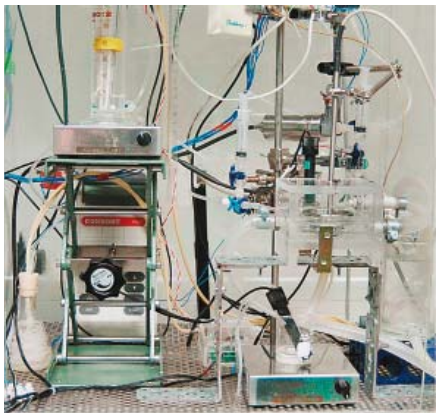
PD Dr. med.
Philipp Dutkowski

Dr. med.
Katarzyna Furrer

Hypothermic oxygenated rat liver perfusion

PD Dr. Philipp Dutkowski, Dr. med. Katarzyna Furrer

During cold storage of livers cellular components deteriorate resulting in energy depletion and loss of function. In addition, the mitochondrial Redox state is affected that greatly reduces the ability of the mitochondrium to transfer energy. Tissue damage from cold storage is further aggravated by rewarming and reperfusion. The combined tissue injury eventually leads to a loss of the graft. Extending or rectifying some of the cellular components might allow to prolong cold ischemia time and may improve the viability of the grafts. In experiments using isolated perfused rat livers, the deterioration of cold stored livers could be demonstrated. A method was developed that could partially rectify this damage: hypothermic oxygenated perfusion (HOPE) is an approach by which the livers can be recharged during the last phase of cold storage. The liver is perfused with an oxygenated, modified UW solution. We could show that machine perfusion improved several parameters including lipid peroxidation, bile flow, energy charge, total glutathione and LDH release. Structurally, these livers exhibited less tissue damage as assessed by electron microscopy. Furthermore, apoptosis was reduced suggesting that central mechanisms of tissue destruction were down regulated.



Isolated rat liver perfusion system. The central part consists of temperature controlled chamber to host the liver. Circulating perfusion fluid is pumped through a reservoir system to keep constant pressure. Sampling of the perfusate and bile production can be accessed any time.

Achievements 2005

Scientific

- Demonstration that HOPE can prevent tissue injury in the explanted liver

Personnel

- PD Dr. P. Dutkowski won an award from the SGC in Zürich

Selected references:

- P. Dutkowski, R. Graf, PA Clavien
  Rescue of the cold preserved liver by hypothermic oxygenated machine perfusion. Am. J. Transplantation 2006 (*in press*).


Dr. med.
Stefan Heinrich


Dr. med.
Felix Dahm


Dr. med.
Daniel Dindo


Dr. med.
Michelle de Oliveira

Oncology

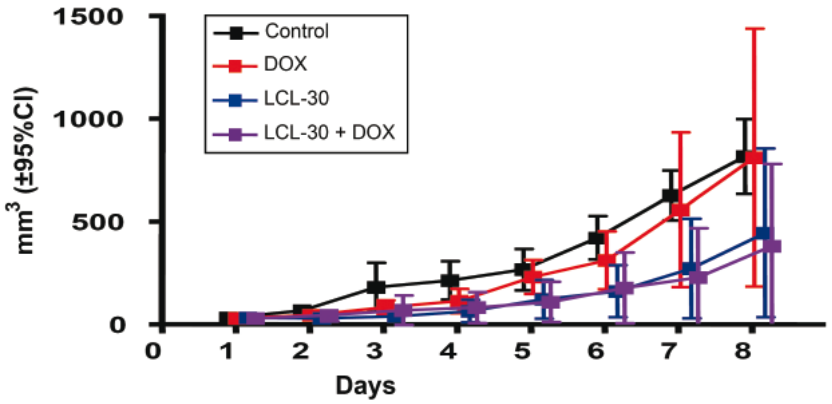
Dr. med. Felix Dahm, Dr. med. Stefan Heinrich, Dr. med. Daniel Dindo, Dr. med. Michelle de Oliveira

Liver metastases of colorectal cancer are frequent indications for liver resections in clinical practice. Liver resection is followed by a regenerative response of the remaining liver. This process of liver regeneration involves growth factors and cytokines which are also implicated in the promotion of tumour growth in-vitro. Therefore the interplay of liver regeneration and colorectal liver and lung metastases was investigated in a mouse model. Animals were injected with a syngeneic colorectal cancer cell line, followed by liver resection (PH) or portal vein ligation (PVL) 7 days later. Liver regeneration and tumour burden were evaluated 7 days after surgical interventions. We found hepatic tumour load to be significantly higher after PVL than after PH, while the growth of lung metastases was not affected by either procedure. We could also show that the presence of liver metastases reduces liver regeneration after resection, while the regenerative response was normal after portal vein ligation. Furthermore we could show that the cytokine TGFβ is produced by tumour cells in-vitro and in tumor tissue in-vivo. This observation might be the explanation for decreased liver regeneration in metastatic disease. These observations have triggered several new experiments investigating the underlying pathomechanisms (Heinrich et al, J.Hepatology 2006, *in press*)

Another project focuses on the role of sphingolipids in cancer cell death and survival. Sphingolipids are a complex class of lipids that not only constitute cellular membranes, but also play an important role in intra- and extracellular signalling. The sphingolipid ceramide plays an important role in the induction of apoptosis. By using liquid chromatography and mass spectrometry we could show that specifically targeted cationic ceramide analogues accumulate in mitochondria in vitro. These compounds exert cytotoxic effects on a variety of cancer cell lines. We studied the human colorectal cancer cell line SW403 in detail and showed a decreased mitochondrial membrane potential, cytochrome c release and cytoplasmic caspase activation. Cells reacted to the ceramide compound by an overall decrease of endogenous ceramide, and a rapid rise of sphingosine-1-phosphate, a sphingolipid counterplayer of ceramide. In vivo we could establish a tolerable dosing regime along with pharmacokinetic behaviour of the ceramide compound. Most importantly, ceramide treatment reduced the growth of subcutaneous tumour metastases in vivo. Targeting mitochondria with ceramide analogues offers a promising new approach to cancer treatment.

In another project we have begun to analyse the effect of chemotherapy on liver morphology and especially on the liver's ability to regenerate after hepatectomy. This study was prompted by the fact that patients with liver metastases receive chemotherapy followed by surgical resection of residual tumours. There is anecdotal evidence to suggest regenerative defects of the liver after chemotherapy.

Treatment of s.c. tumours



Assessment of tumour growth of mice treated with either Doxicycline, with a long chain ceramide analogue (LCL-30) or with a combination of both.

Achievements 2005

Scientific

- Colorectal cancer cells reduce liver regeneration by producing TGFβ
- Dose-finding, pharmacokinetics and proof of efficacy of a long-chain ceramide analogue in vivo

Personnel

- Dr. D. Dindo received an award for his lecture at the annual meeting of the Swiss Society of Surgery.
- Dr. F. Dahm received a grant from the Sassella Foundation.
- Dr. S. Heinrich received the Research Prize of the Swiss Surgical Society and a grant from the Cancer League of Zurich.

Collaborations:

- Prof. Dr. Y.A. Hannun and Prof. Dr. A. Bielawska, Department of Biochemistry and Molecular Biology, University of South Carolina, Charleston, USA
- PD Dr. W. Jochum, Department of Pathology, University Hospital Zurich

Selected references:

- Selzner M, Bielawska A, Morse MA, Rüdiger HA, Sindram D, Hannun YA, Clavien PA. Induction of apoptotic cell death and prevention of tumor growth by ceramide analogues in metastatic human colon cancer. Cancer Research (2001)
- Selzner N, Selzner M, Graf R, Ungethuem U, Fitz JG, Clavien PA. Water induces autocrine stimulation of tumor cell killing through ATP release and P2 receptor binding. Cell Death and Differentiation (2004)

2.2.2 Islet Cell Laboratory

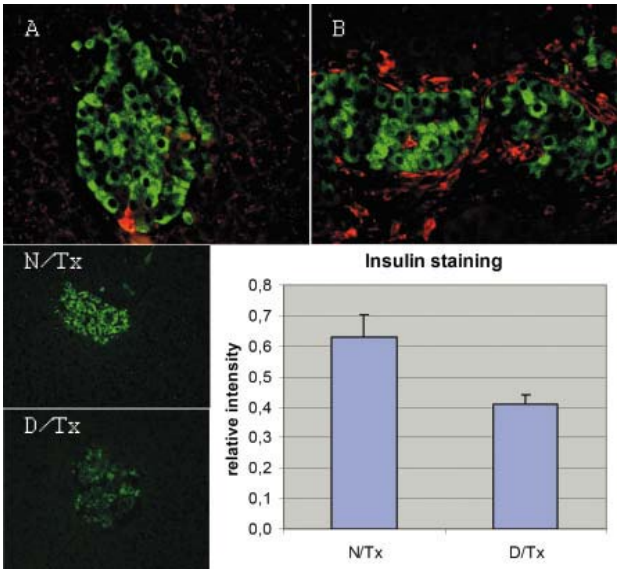
PD Dr. med.
Markus Weber

Dr. phil. II
Wolfgang Moritz

Lu Tuyet Trinh

The fate of grafted islets in the immediate posttransplantation period
PD Dr. med. Markus Weber, Dr. Wolfgang Moritz

Our main research interest is focused on islet cell transplantation in particular to develop strategies to improve its efficacy and is performed in close collaboration with the Endocrinolology and Diabeteolgy Unit of the University Hospital. For the last five years, islet transplantation has become a widely used therapy for patients with type 1 diabetes mellitus. Islets are isolated from pancreata of heart-beating donors and transplanted into the liver of diabetic recipients by transhepatic portal catherization. Unfortunately, the requirement of donor tissue is quite high (2-3 pancreata) in order for a diabetic patient to become insulin independent. The reasons for such a high demand are manifold; insufficient isolation efficiency and graft rejection are certainly to mention. It has been suggested, that due to their devascularized state, transplanted islet would potentially suffer from near-ischemic conditions and consequently undergo massive cell death. We therefore are investigating strategies to interfere successfully with the deleterious effects of hypoxia and nutrient deprivation for the immediate posttransplantation period until complete revascularization of the transplant has taken place.



Rat islet after syngeneic, intrahepatic transplantation: (A) Selective loss of glucagon expressing alpha-cells (red). (B) Microvasculature (red) predominantly formed at the islet's periphery. Insulin staining (green) of the islet graft is markedly decreased in diabetic rats (D/Tx) when compared to normoglycemic recipients (N/Tx).

Achievements 2005

- In order to evaluate the fate of intrahepatically transplanted islets, we performed a series of syngeneic islet transplantation in diabetic rats.
  By the choice of a subtherapeutic islet mass we aimed to simulate transplantation of marginal donor tissue. As opposed to in vitro studies under hypoxic conditions, histological examination of islet grafts did not reveal obvious signs of massive tissue loss, nor signs of cell death by necrosis or apoptosis. This was also confirmed by the assessment of total insulin positive beta-cell area in histological sections, which did not change in the course of the first 14 days after transplantation. However, we observed a selective loss of glucagon producing alpha-cells, while graft revascularization was predominantly confined to the islet periphery and accompanied by strong stroma formation. Cellular insulin content seemed to be decreased when compared to native pancreatic islets or islets transplanted into normoglycemic recipient rats. Post transplantation insulin treatment improved graft performance which was paralleled by restored insulin stores. Despite their initial avascular state, intrahepatically transplanted islets do not undergo substantial cell death but rather loose their secretory capacity due to exhaustion. Hence, islet graft function is negatively affected by the diabetic state and therefore largely dependent on the maintenance of normoglycemia, particularly in the immediate early phase after transplantation. Our observations underline the need of a tight blood glucose control post-transplantation, especially when only marginal, subtherapeutic graft tissue was available for transplantation.
  This project is supported by the Swiss National Science Foundation. Additional funding has been received by the Olga-Mayenfisch, Hartmann-Müller and the Hermann-Klaus Stiftung.

Collaborations:

- The research project is also part of a close collaboration with PD Dr. Lehmann and Dr. Züllig of the Unit of Endocrinology and Diabetology in Internal Medicine of the University Hospital Zürich and Prof. Max Gassmann of Veterinary Physiology of University of Zürich.

Selected references:

- Moritz W., Meier F., Stroka D. M., Giuliani M., Kugelmeier P., Nett P.C., Lehmann R., Candinas D., Gassmann M., Weber M. (2002) Apoptosis in hypoxic human islets correlates with HIF-1 alpha expression. FASEB J. 2002 May; 16:745-7
- Giuliani M, Moritz W, Bodmer E, Dindo D, Kugelmeier P, Lehmann R, Gassmann M, Groscurth P, Weber M. Central necrosis in isolated hypoxic human pancreatic islets: Evidence for post-isolation ischemia. Cell Transplant: 14: 67-76, 2005

2.2.3 Pancreatitis Research Laboratory

PD Dr. phil. II
Rolf Graf

PD Dr. med.
Daniel Bimmler

Dr. med.
Li K. Sun

Dr. rer. nat.
Franco Fortunato

Dipl. phil. II
Theresia
Reding Graf

Martha Bain

cand.med.
Federico
Storni

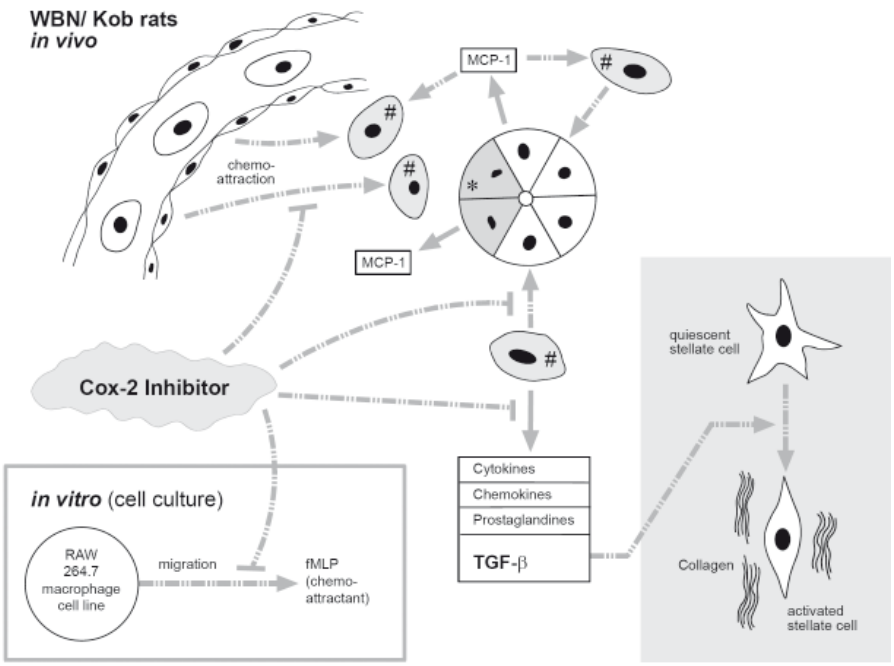
PD Dr. Rolf Graf, PD Dr. med. Daniel Bimmler

The role of COX-2 in chronic pancreatitis
Dr. med. Li-Kang Sun, Martha Bain, Dipl. phil. II Theresia Reding Graf, cand. med. Federico Storni

Our laboratory has a long standing interest in the pathophysiology of chronic pancreatitis.
We used a spontaneous model of pancreatic inflammation and fibrosis (WBN/Kob rat) to test whether a COX-2 inhibitor reduces inflammation and fibrosis. During the early phase of pancreatitis, inflammatory cells increase dramatically in untreated rats while in COX-2 inhibitor fed rats they were reduced in number. One of the predominant cell types, the macrophage, was significantly less abundant in treated rats and appears to affect the course of pancreatitis. Concomitant activation of pancreatic stellate cells was strongly inhibited suggesting that pancreatic fibrosis is targeted by the COX-2 inhibitor. Parameters of acute and chronic inflammation were significantly down regulated e.g. TNF-α, Il-6 MCP-1 and TGF-β.

These observations led us to test whether COX-2 might be involved in chemotaxis. Therefore, we performed cell culture experiments to demonstrate that prostaglandins (down-stream products of COX-2) modify the behaviour of macrophages. In chemotaxis chambers we show that the directional response of a mouse macrophage cell towards a chemotactic signal was strongly inhibited in the presence of a COX-2 inhibitor.

The relationship of COX activity (PGE_2) with macrophage recruitment in pancreatitis was further investigated. MCP-1, a chemoattractant, was found predominantly in pancreatic acinar cells in the rat model. To simulate interactions of macrophages and acinar cells, we used a mouse macrophage cell line (RAW 264.7) and a rat acinar cell line (AR42J). We then verified that PGE_2 receptors $EP_{1\text{-}4}$ are actually expressed in AR42J cells. The expression of MCP-1 was regulated by TNFα and PGE_2 in pancreatic cells. In the presence of PGE_2, TNFα dependent expression of MCP-1 was significantly higher than with TNFα alone. TNFα regulated the expression of itself in this cell line. In the presence of PGE_2, expression levels of TNFα were further enhanced. Activated macrophages induced the secretion of pancreatitis-associated protein in the AR42J cell. The presence of acinar derived molecules caused a markedly increased directional migratory response in macrophages.
We conclude that the chemical interactions of macrophages and acinar cells might be a key step in establishing the chronic phase of pancreatitis.



Acinar cell damage (*) leads to the nearby accumulation of activated macrophages (#) due to chemoattraction, e.g. exerted by MCP-1 which is secreted in the vicinity of injured acinar cells.
Activated macrophages produce and shed various cytokines, chemokines, prostaglandines and TGF-β. The latter stimulates the activation of stellate cells which consequently synthesize and deposit collagen fibers, eventually leading to fibrosis.
COX-2 inhibitors interfere with different processes: they inhibit chemoattraction and the secretory activity of macrophages, especially the secretion of TGF-β.
In our WBN/Kob rats we have shown that infiltration of macrophages and subsequent fibrosis are significantly reduced and delayed if rofecoxib, a COX-2 inhibitor, is administered.
Inset: In vitro experiments using a macrophage cell line (RAW 264.7) demonstrated a similar effect of rofecoxib: the directed migration of macrophages caused by the chemoattractant fMLP was significantly reduced with rofecoxib.
We therefore hypothesize that the infiltration of activated macrophages represents an essential step in the pathogenesis of chronic pancreatitis.
Full arrow: substances secreted by a cell. **Broken arrows**: positive regulation. **Line with a dash at the end**: negative regulation.

Acute pancreatitis in the alcoholic pancreas

Dr. Franco Fortunato

Alcohol is an important factor in the etiology of pancreatitis. To assess whether alcohol modifies the response to noxious stimuli, we analyzed the pancreata derived from animals after chronic alcohol feeding and a single dose of LPS (lipopolysaccharide). There was a dose dependent response of tissue injury in animals injected with LPS. Concurrent alcohol feeding aggravated the response. We could show that apoptosis was significantly reduced in alcohol fed rats. In the presence of LPS, this was further inhibited. We conclude that inhibition of apoptosis after alcohol exposure may lead to a switch to necrotic cell death. This in turn might activate a stronger inflammatory reaction, which we could demonstrate by quantification of macrophages (Fortunato et al, Am. J. Physiol 2006, *in press*).

Achievements 2005

Scientific

- We have been able to show that chemical signals from macrophages and acinar cells promote chronic activation and may represent a mechanism of chronic inflammation and fibrosis.
- COX-2 is involved in macrophage recruitment.
- Apotosis is inhibited by alcohol and tissue injury is switched to a necrotic type of cell death. Publication in Am. J. Physiology.

Collaborations:

- Dr. Aurel Perren, Institut für klinische Pathologie, Universitätsspital Zürich
- Dr. Pia März & Prof. U. Otten, Physiologisches Institut, Universität Basel
- PD Dr. Marius Keel & Dr. Luc Härter, Klinik für Unfallchirurgie, Universitätsspital Zürich
- Dr. Martin Hersberger, Institut für Klinische Chemie, Universitätsspital Zürich
- Dr. George Scheele, Institute of Genomic Medicine, LaJolla, Ca. USA
- Dr. Robert DeLisle, Anatomy and Cell Biology, University of Kansas School of Medicine, Kansas City, KS

Selected references:

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- Reding TV, Bimmler DR, Perren A, Sun LK, Fortunato F, Storni F, Graf R. A selective COX-2 inhibitor suppresses chronic pancreatitis in an animal model (WBN/Kob rats): significant reduction of macrophage infiltration and fibrosis. Gut 2005 (epub ahead of print)
- Fortunato F, Deng X, Gates LK, McClain CJ, Bimmler D, Graf R, et al. Pancreatic Response to Endotoxin after Chronic Alcohol Exposure: Switch from Apoptosis to Necrosis? Am J Physiol Gastrointest Liver Physiol 2005. (epub ahead of print)

2.2.4 Colorectal Surgery

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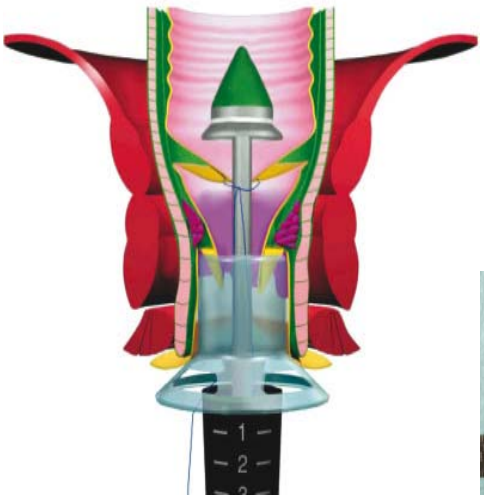
Clin. Ass. Prof.
Dr. med. Dieter
Hahnloser

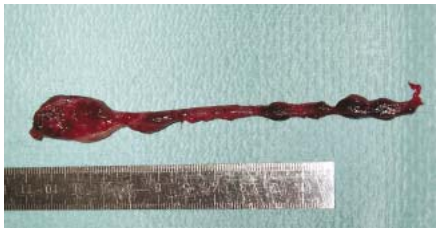
Dr. med. L. Sun

Dr. Franc Hetzer MD, Dr. Dieter Hahnloser MD, Dr. L. Sun, MD,
PD Dr. Nicolas Demartines

Haemorrhoids are a frequent and disturbing benign disease of the anus. The pathogenesis of haemorrhoids is multifactorial including a stasis in the haemorrhoidal venous plexus causing inflammation and dilatation of the venous membrane and resulting in chronic bleeding, itching and pain. The inflammation is triggered by intracellular- (ICAM) and vascular cell adhesion molecules (CVAM) followed by liberation of prostaglandines. Flavinoid (Daflon©) inhibits these adhesion molecules and therefore reduces prostaglandine levels. In vitro and animal studies have demonstrated increased vasoconstriction of the venous plexus, reduced capillary leakage and reduced prostaglandine levels E_2, $F_{2\alpha}$ and thromboxan B2 after Daflon©. Several clinical studies have shown that Daflon© significantly reduces the classical symptoms of acute and chronic bleeding.

We are investigating in a prospective randomised trial of patients requiring hemorrhoidectomy the influence of Daflon© perioperatively vs. placebo on the prostaglandine concentration within symptomatic haemorhhoids. We hope to demonstrate the local effect of orally taken Daflon© on prostaglandine synthesis in hemorrhoidal tissue and therefore to elucidate the role of prostaglandines in the pathogenesis of this frequent disease.

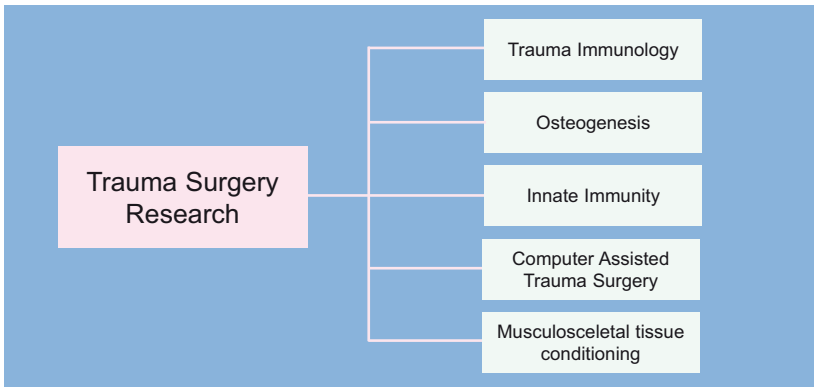




2.3 Trauma Surgery Research


PD Dr. med.
Marius Keel


Prof. Dr. med.
Otmar Trentz



2.3.1 Trauma Immunology


PD Dr. med.
Marius Keel


Dr. rer. nat.
Luc Härter


Dr. med.
Ludwig Labler


Dr. med.
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Neutrophil activation and accelerated angiogenesis in vacuum assisted closure-treated wounds after trauma
PD Dr. med. Marius Keel MD, Dr. rer. nat. Luc Härter

Wound healing is a complex chronologically and spatially organized process initialized by a local inflammation and infiltration of neutrophil granulocytes (PMN) and macrophages. These infiltrating leukocytes set the stage for later processes which finally lead to remodeling of the vasculature and the affected tissue. The management of large, possibly infected wounds poses a major challenge in trauma surgery. In the past many different methods of temporary wound closure have been exploited. A current standard is the wound cover by Epigard® and the Vacuum Assisted Closure (V.A.C.®) system. The V.A.C.®-sytem is a temporary wound cover with a polyvinyl foam sealed with a polyurethane foil. In the clinical practice the V.A.C.®-therapy shows a quantitative and qualitative increase of granulation tissue formation on the wound surface. The mechanisms leading to this improved wound healing are still not known. The increased microvessel density which is seen later most possibly reflects the improved granulation formation and wound healing. Our hypothesis is that V.A.C.®-therapy promotes a better and accelerated wound healing by an accumulation and activation of PMN at the wound site with an induction of endothelial cell proliferation and angiogenesis. This accelerated angiogenesis is promoted by mediators released from activated PMN.
We investigated the participation of neutrophil granulocytes in initialization and propagation of the early wound inflammation and angiogenesis during V.A.C.®-therapy after trauma. Several parameters such as expression of integrins, generation of oxygen radicals, release of cytokines and apoptosis are analyzed in PMN and fluids isolated from the patient's wound and compared with serum and systemic PMN isolated from peripheral blood. The release of different mediators was measured in ELISA.
From 58 patients serum and wound fluid samples (n=122) were obtained. Cytokines and growth factors (IL-6, IL-8, IL-10, VEGF, FGF-2) were measured

by ELISA. Significantly higher levels for IL-8 and VEGF were observed in woundfluids of VAC-treated patients with polytrauma, whereas other mediators remained unchanged. The increased level of IL-8 and VEGF could enhance inflammation and angiogenesis and thus might improved wound healing.

Understanding the mechanisms involved in the wound healing during V.A.C.®-therapy might enable timely addition of healing-promoting factors to the wound ground in the future. This could further improve the healing processes, especially in infected wounds, or in wounds from severely injured patients suffering from an essential immunosuppression during the post-traumatic course.

Achievements 2005

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Collaborations:

- PD Dr. R. Graf, Division of Visceral & Transplant Surgery, USZ, Zürich
- Prof. Dr. D. Demitriades, Director of Trauma / Surgical Critical Care, University of Southern California, Los Angeles, USA

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- Marius Keel, Ludwig Labler, Otmar Trentz „Damage Control“ in Severely Injured Patients: Why, When, and How? European Journal of Trauma 2005;Vol 31(3):212–221

2.3.2 Osteogenesis Laboratory & Bone Research


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Egermann


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Alexander E.
Handschin


Dr. med.
Omana A.
Trentz


Dr. med.
Markus Cardell


Sonja Hemmi

PD Dr. med. G. Wanner, Dr. P. Richards, Dr. med. O. A. Trentz,
Dr. med. M. Egermann, Dr. med. A. E. Handschin, S. Hemmi

Fracture Healing in Osteoporosis

Osteoporosis, a major public health burden, is associated with increased fracture risk. Fracture healing in osteoporosis is altered with reduced callus formation and impaired biomechanical properties of newly formed bone leading to high risk of fixation failure. Fracture healing in an established small animal model for osteoporosis (SAMP-6 mice) is investigated using a newly developed angular stable fixation device. Analysis is performed using histology, computed tomography and biomechanical testing.
Bone marrow-derived mesenchymal stem cells (BMSC) provide an alternative to bone grafting and composites enriched in BMSC demonstrated superior bone healing. Matrices enriched in BMSC may increase the bone repair in osteoporosis-associated fractures. Although BMSC from osteoporotic donors have shown decreased proliferation and osteoblastic differentiation pattern in-vitro, it is unclear whether BMSC from osteoporotic individuals possess similar healing capacities in-vivo like BMSC from healthy donors. Furthermore it is unknown if BMSC from osteoporotic donors could be stimulated to increase their proliferation and differentiation pattern. In addition we study the influence of BMSC from osteoporotic and healthy donors on bone healing.

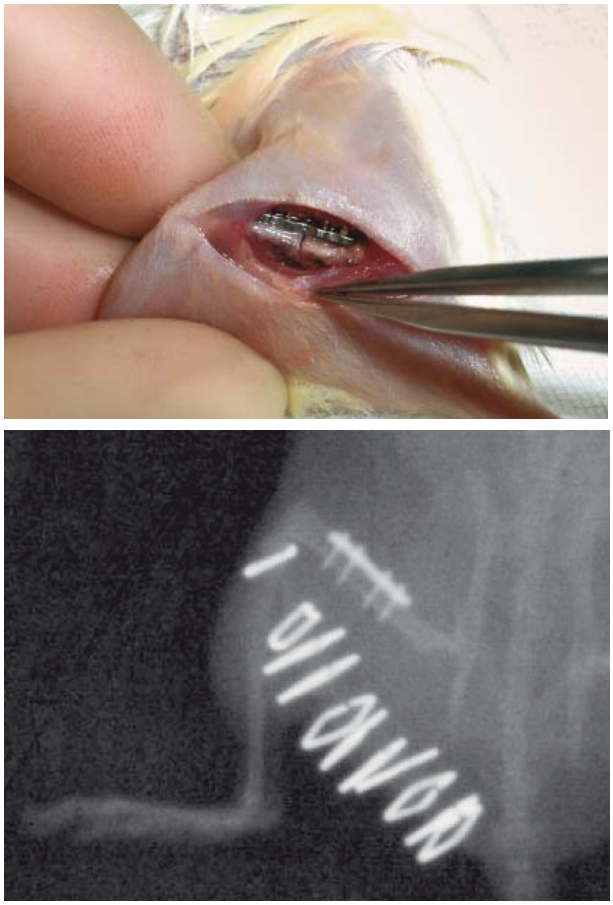

Figure 1: Newly developed angular stable implant fixing a midshaft osteotomy in osteoporotic mice.

Effect of leptin on bone cells

Leptin, known as the obesity gene, is playing a crucial role in maintaining bone turnover. In leptin knock out mice, the infusion of intraventricular recombinant leptin into the hypothalamic area leads to a massive induction of osteoporosis. In contrast to this central effect, there is also a hypothesis on a direct, peripheral effect of leptin on bone cells. To further elucidate this hypothesis we are currently investigating the effect of recombinant leptin on primary human osteoblasts in vitro. One of our working hypothesis is that leptin may affect the late mineralization stage of mature osteoblasts, enhancing the mineralization of the extra cellular matrix.

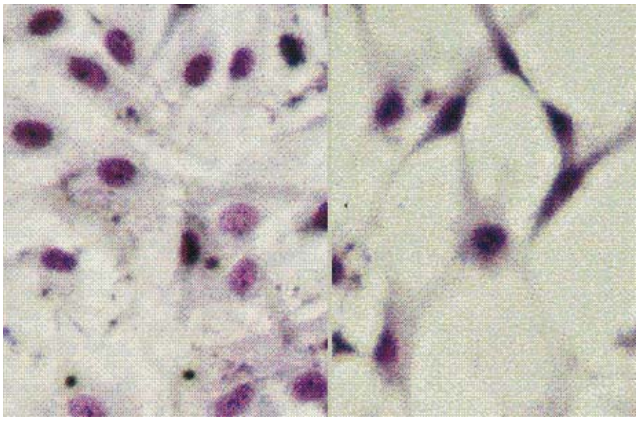

Figure 2: Human osteoblast after 21 days of incubation under leptin influence (left) and without (right).

Bone microtissue

During the last years, culturing of osteoblasts has been extensively studied in this lab. The biochemical behaviour of cells attached to standard culture devices doesn't represent physiological processes in detail. Our approach of bone tissue engineering includes a newly developed method of three-dimensional cell growth. This method lacks any scaffolds since they interfere with cell metabolism. The planned studies, in collaboration with Professor Hoerstrup, will examine the potential of osteoporotic BMSC to form mineralized micro-tissue using the hanging drop technique. The ultimate goal of which is to establish a multifunctional "microbone" in vitro which maybe used to enhance our understanding of the mechanisms involved in bone disease.

Achievements 2005

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Collaborations:

- AO Research Institute, Davos, Switzerland (Prof. K. Ito, Prof. M. Alini)
- AO Development Institute, Davos, Switzerland (R. Matthys)
- Departement of Clinical Research, University of Berne (Prof. W. Hofstetter)
- Division of Regenerative Medicine, Department of Surgical Research, University Hospital of Zurich (Prof. S. Hoerstrup, Dr. J. Kelm)
- PD Dr. med. HJ Kock, Unfallchirurgie, Hochtaunuskliniken Bad Homburg

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2.3.3 Innate Immunity Laboratory

PD Dr. med.
Michael
Heinzelmann

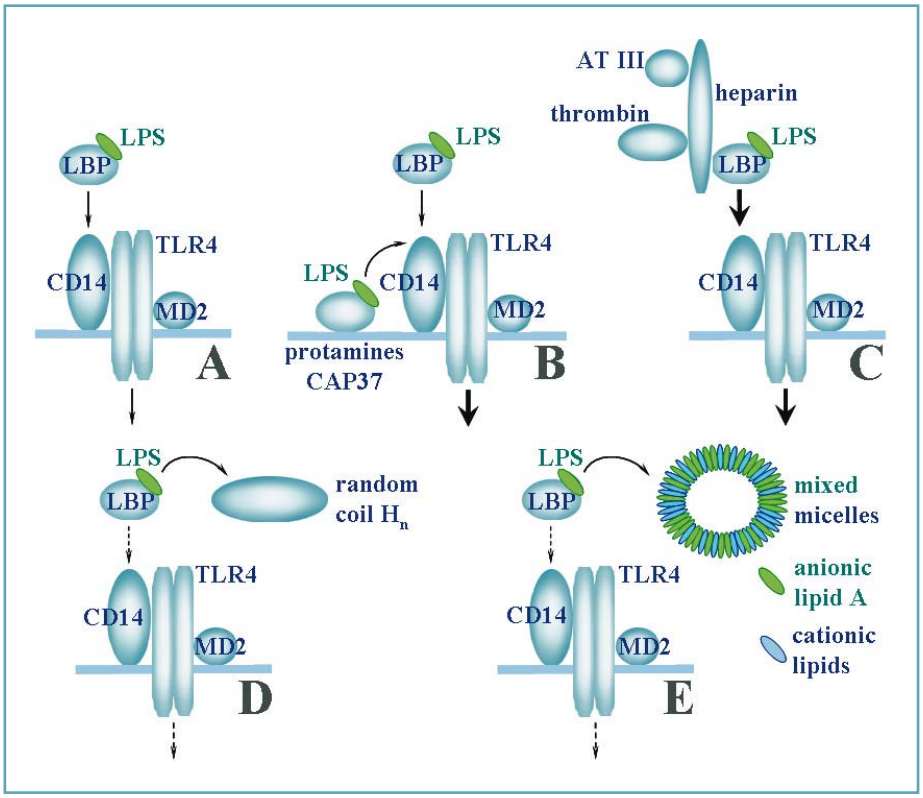
Dr. med.
Herbert Bosshart

Targeting Bacterial Endotoxin
Michael Heinzelmann & Herbert Bosshart

The term sepsis describes a potentially lethal clinical condition that develops as a result of a dysregulated host response to bacterial infection. The commonest bacterial component implicated in initiating the septic syndrome is a cell wall molecule derived from Gram-negative bacteria, known as lipopolysaccharide (LPS) or endotoxin. Like all mammals, humans are equipped with an LPS-sensing machinery consisting, primarily, of LPS-binding protein (LBP), CD14, a glycosylphosphatidylinositol (GPI)-anchored monocyte differentiation antigen, and Toll-like receptor 4 (TLR4), a signal transducing integral membrane protein. Modest stimulation of TLR4 facilitates the elimination of invading microorganisms. Potent TLR4 stimulation, however, produces severe reactions in the host, often leading to multiple organ failure and death. The search for pharmaceuticals that reduce mortality in septic patients has been a painstaking process. Thus far, only a few compounds have been found to significantly reduce mortality rates. Perhaps one of the more promising therapeutic strategies currently pursued is based on the identification of synthetic or naturally-occurring substances that neutralize LPS or inhibit LPS-mediated activation of host immune cells such as monocytes and macrophages. Over the past few years, we have identified a number of molecular structures with a capacity to either enhance or blunt LPS-induced monocyte activation.

Our studies and those of others have uncovered several mechanisms by which LPS-induced inflammatory responses in human whole blood are modified. We showed that binding of LPS to cationic polypeptides can result in both, enhancement or inhibition of LPS signals, depending on the type of amino acid that contributes most to the cationic nature of the polypeptide. Arginine- and lysine-rich cationic polypeptides are enhancers of LPS signals. Histidine-rich polypeptides, in their protonated state, bind and neutralize LPS. Cationic lipids act as LPS neutralizers by entrapment of LPS in micelles. Sulfated polysaccharides like heparin or dextran sulfate associate with LBP, displace LPS, and accelerate the transfer of LPS to other acceptor molecules, e.g. soluble or membrane-bound CD14, resulting in an enhanced inflammatory response of monocytes, and, presumably also of endothelial cells.

The molecular mechanisms of LPS enhancement and inhibition are of immediate clinical relevance. The immunologically inert anticoagulant fondaparinux can be used as a replacement for LPS-enhancing heparin, at least in patients who are at risk for developing Gram-negative infections. Protamines, although strong enhancers of LPS responses, are probably safe, as long as patients are not treated with this polycation repeatedly. LPS-inhibiting histidine-rich peptides, or cationic lipids are promising compounds for future therapeutic interventions in patients with sepsis. However, for these and many other LPS-neutralizing substances, bridging the gap between bench and bedside, may not be a straightforward process.



Models for the interaction of LPS-enhancing or -inhibiting substances with extracellular components of the LPS activation pathway. (A) In human plasma, circulating LPS-LBP complexes are recognized by monocytic CD14 (top vertical arrow). MD2-dependent activation of TLR4 initiates a signaling cascade that results in the production and release of several proinflammatory mediators (bottom vertical arrow). Alternatively, LPS bound to soluble CD14 activates endothelial cells which express TLR4 and MD2 but not CD14. (B) Cationic proteins, e.g. protamines and CAP37, associate with LPS and bind to cellular surfaces. Subsequent steps (curved arrow) leading to an enhanced LPS response (bottom vertical arrow) have not yet been addressed experimentally. (C) Heparin exerts its anticoagulant effect through binding to AT III and thrombin. Apart from its interaction with proteins of the coagulation pathway, heparin binds to LBP and displaces LPS (top vertical arrow) resulting in enhanced LPS signals (bottom vertical arrow). (D) Poly-L-histidines (Hn) are devoid of stable secondary structures, i.e. they exist as random coils. Similar concentrations of LBP and Hn result in near complete inhibition of LPS responses in human whole blood (dashed arrows), presumably through adsorption of LPS to Hn (curved arrow). (E) Cationic lipids neutralize LPS responses (dashed arrows) by incorporating LPS into micelles (curved arrow).

Achievements 2005

Published Work

- Heinzelmann M, Bosshart H (2005). Heparin binds to lipopolysaccharide (LPS)-binding protein, facilitates the transfer of LPS to CD14, and enhances LPS-induced activation of peripheral blood monocytes. J Immunol, 174: 2280-7.

Lectures

- Bosshart H, Modulation of Host Responses to Bacterial Endotoxin. International Conference on Environmental, Industrial and Applied Microbiology, March 15 18, 2005, Badajoz, Spain.

Collaborations:

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Selected references:

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2.3.4 Computer Assisted Trauma Surgery (CATS)

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Dr. med.
Valentin Neuhaus

Dipl. Ing. FH
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PD Dr. med. Marius Keel, Dr. med. Felix Matthews, Dipl. Ing. FH Adrian John,
Dipl. Inf. Ing. ETH Adrian Egli, Dr. med., med. dent. Heinz-Theo Lübbers

Computer assisted surgery has started with navigation about 15years ago in neurosurgery and soon later in ENT. PD Dr. Wanner and PD Dr. Keel introduced 2D navigation early in 2002 into our clinical work after a phase of training and precision studies. With the liquidation of Medivision and transition via Praxim to BrainLab there was a long period with no technical support for laboratory and clinical projects in computer assisted surgery. Only in March 2005 the new fluoro navigation system of BrainLab became functional. So we used the time to build a network of cooperations with the CARCAS Research Group of the University Hospital of Basel, the AO Development Institute (ADI) in Davos and some new industrial partners, such as Siemens Switzerland and Siemens World (Erlangen, D), BrainLAB (Heimstetten, D) and Synthes (Oberdorf, CH). Together with these partners we expanded our research field from navigation to technology integration of different aspects and intraoperative imaging in trauma. Siemens and BrainLab could be linked to our university with contracts within different projects.
Together with AO-International (AOI), BrainLab and other partners from the Computer-assisted Surgery Expert Group of the AO (University of Ulm, BG Klinikum Ludwigshafen) teaching modules with workshops, videos and posters were developed.
With a new software for navigated positioning of the fluoroscope precision tests have been performed. They are under evaluation and preparation for publication. Clinical tests will follow.
Another new software for navigated reduction control in maxillo-facial surgery has been installed on the trauma navigation system and successfully been used intra-operatively in 5 cases (Fig 1a+b) (Dr. Lübbers).

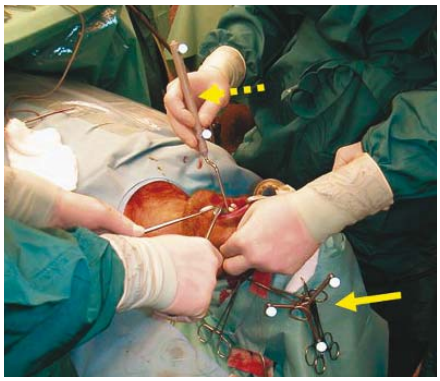

Figure 1a
Navigated control of correct reduction of the zygoma fracture. Full arrow shows dynamic reference base on the skull. Dotted yellow arrow shows referenced pointer

Figure 1b
Display which shows the planning and the actual positon of the bone after reduction.

Laboratory tests of stability of a passive holding device for computer aided reduction and feasibility for intra-operative use of the device were published this year (see publication record).
Together with Siemens, Synthes and CARCAS we are working on a software solution (i3db) to improve workflow from admittance of the patient to pre-operative planning (fig 2), intra-operative handling, optimization of storage, documentation, and accounting. The project involves different divisions of the hospital. Meetings with OR personnel from several hospitals were hold. A large KTI-grant application was accepted, so that we will employ a further ETH - IT researcher in March 2006. This project is actually the most comprehensive one of our research group.
Several other research grants have been written and accepted.

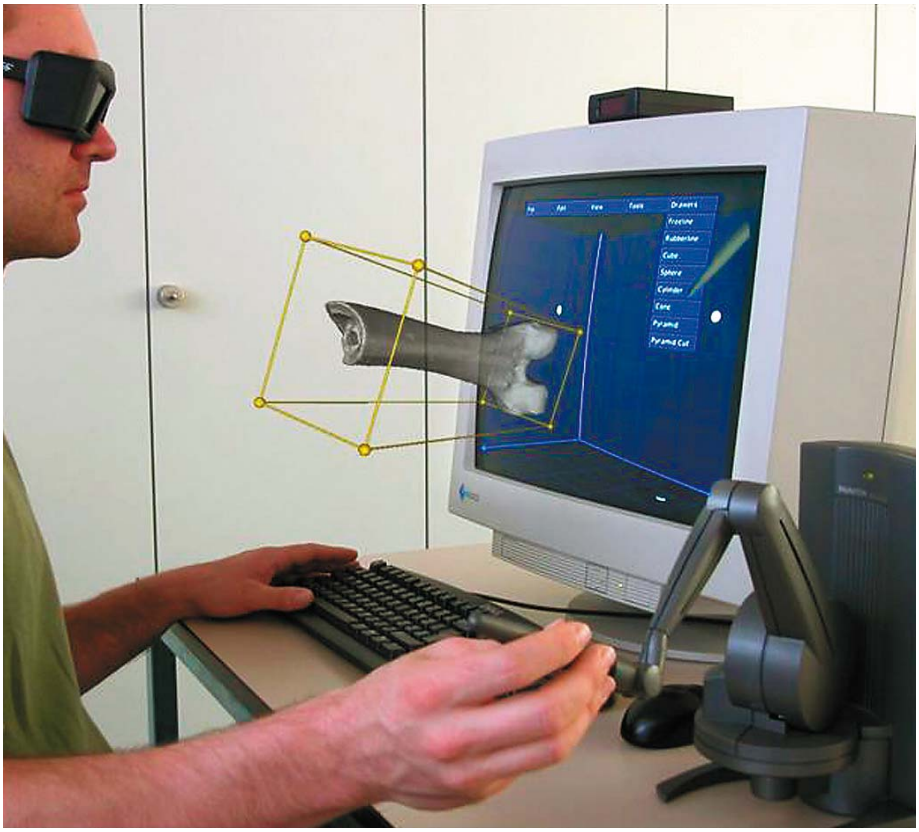

Figure 2
Virtual 3D planning and simulation

Achievements 2005

- Establishment and consolidation of cooperation with industrial partners: Siemens, BrainLab, Synthes
- Cooperation with CARCAS Basel continued
- MaxFax navigation software of BrainLAB installed and used.
- MEPUC project: Precision testing performed
- I3db workflow project supported by a large KTI grant.
- Stability tests of passive holding device published.

Collaborations:

- PD Dr. Simon Wildermuth, Institut für Diagnostische Radiologie, Universitätsspital Zürich
- Prof. Grätz, Dr. R. Eglmeier, Dr. H. Lübbers, Klinik und Poiklinik für Kiefer- und Gesichtschirurgie, Universitätsspital Zürich
- Dr. D. Holzmann, Klinik für Ohren-, Nasen-, Hals- und Gesichtschirurgie, Universitätsspital Zürich
- CARCAS, Research Group for Computer Assisted Radiology and Surgery of the University Hospitals of Basel and Zurich
- Expert Group Computer Assisted Surgery of the AO Foundation with the following partners:
  - Prof. C. Krettek, Unfallchirurgische Klinik, Medizinische Hochschule Hannover MHH
  - Prof. F. Gebhard. Abteilung für Unfallchirurgie, Hand- und Wiederherstellungs-chirurgie, Universitätsklinikum Ulm
  - Prof. J. Alonso, Orthopaedic Trauma Service, University of Birmingham, Alabama
  - PD Dr. P.A. Grützner, Klinik für Unfall und Wiederherstellungschirurgie, BG Unfallklinik Ludwigshafen
  - PD Dr. U. Stöckle, Centrum für Muskuloskeletale Chirurgie, Charité, Universtätsmedizin, Berlin
- AO Development Institut, Davos
- University of Applied Sciences Bern/Biel
- Siemens Med, Erlangen, Deutschland
- Siemens Schweiz, Zürich Altstetten
- BrainLab, Heimstetten, Deutschland
- Synthes, Solothurn

Selected references:

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- Felix Matthews, Valentin Neuhaus, Daniel Schmucki, Ronald Schwyn, Thomas Gross, Pietro Regazzoni, Otmar Trentz, Peter Messmer: Passive Pneumatic Stabilization Device for Assisting in Reduction of Femoral Shaft Fractures. European Journal of Trauma, No 6, Vol 31, 568-574, December 2005
- Hügli RW, Staedele H, Messmer P, Regazzoni P, Steinbrich W, Gross T: Displaced Anterior Column Acetabular Fracture : Closed Reduction and Percutaneous CT-navigated Fixation. Acta Radiol. 2004 Oct. 45 (6): 618-21

2.3.5 Musculosceletal tissue conditioning

PD Dr. med.
Guido Wanner

Dr. med.
Claudio Contaldo

Ahmed Elsherbiny
Wiss. Mitarbeiter

Andrea Schleh
Wiss. Mitarbeiterin

Effects of recombinant Erythropoietin on critically perfused hamster skin flap

Dr. med. Claudio Contaldo; Ahmed Elsherbiny, PD Dr. med. Guido A. Wanner

Originally, stimulation of erythrocytic progenitors was thought to be the only physiological function of Erythropoietin (EPO). But EPO seems to have cytoprotective actions as well as vascular functions. It has been reported that survival of hypoxic tissue may be improved after EPO-treatment. The aim of the present study was to assess the effect of systemically administered EPO on the microhemodynamics in critically perfused skin flap tissue and to investigate the mechanism of protection. The experiments were performed in the hamster dorsal pedicled island flap model **(Fig. 1)**, which consists of a proximal anatomically perfused area, and a distal ischemic part **(Fig. 2)**. By use of intravital fluorescence microscopy the effects of intraperitoneally administred Recormon (either 500 or 5000 IE/ kg bw) on the hypoxic microvasculature in the flap were investigated up to 5 hours after onset of ischemia. To detect a possible mechanism of action eNOS was blocked by L-NAME. In the ischemic flap area of control (n=6) animals after 5 hours of ischemia, nutritive perfusion represented by the functional capillary density (FCD) was decreased to 51 ± 2% ($p < 0.01$ vs.BL, capillary diameters(CD) were dilated to 141 ± 2% ($p < 0.01$ vs.BL) and capillary volumetric bloodflow (VBF)was diminished to 32 ± 5%($p < 0.001$ vs.BL) of baseline values. This microcirculatory dysfunction was substantially attenuated by pretreatment with EPO 5000 IE/ kg bw (n=6); FCD 72 ± 8%, CD 119 ± 2%, VBF 64 ± 8% of baseline (for all parameters $p < 0.01$ vs.Control). The lower dose of EPO 500 IE/ kg bw showed an almost equal effectiveness (n=6); FCD 64 ± 9%, CD 122 ± 3%, VBF 50 ± 11% (for all parameters $p < 0.01$ vs.control, and for VBF; $p < 0.05$ vs. EPO 5000 IE/ kg bw). The beneficial effects of EPO were abolished when L-NAME was administred (n=6); FCD 53 ± 4%, CD 140 ± 3%, VBF 34 ± 6% (for all parameters $p < 0.01$ vs.control). In the same time we observed a significant reduction of apoptotic cells in the dermal layer after EPO treatment **(Fig. 4)** compared to control animals **(Fig. 3).** From our results we conclude that Erythtropoietin may be used as a cytoprotective substance with direct beneficial effects on the microvasculature in critically perfused tissue., which may be mediated by e-NOS. The fact, that the recombinant human form of EPO is a widely used drug for the treatment of anaemia with a well-known safety profile, makes it possible to use it soon in clinical trials.

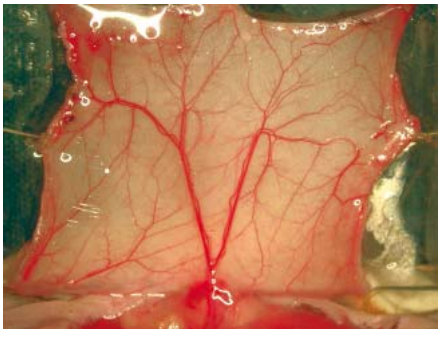

Figure 1 Hamster flap

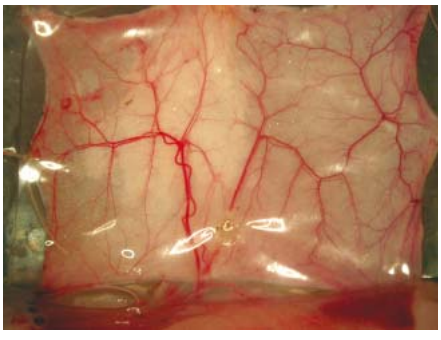

Figure 2 Anatomic and ischemic part

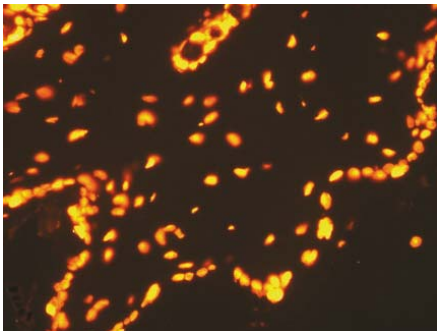

Figure 3 TUNEL positive cells in control skin

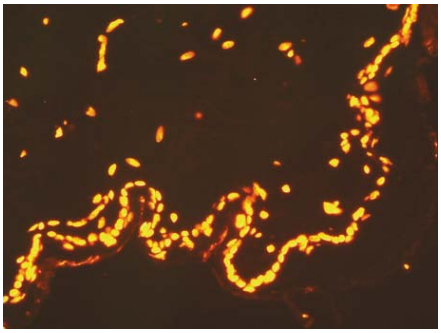

Figure 4 TUNEL positive cells after EPO treatment

Achievements 2005

- Forschungspreis 2005 der Schweizerischen Gesellschaft für Plastische, Rekonstruktive und Aesthetische Chirurgie (SGPRAC) für Dr. C.Contaldo

Talks 2005

- Erythropoietin-Doping in der elektiven Weichteilchirurgie? Contaldo C, Trentz O, Menger MD, Wanner GA 122. Deutscher Chrirugenkongress (München, 05.04-08.04.05)
- Local heat preconditioning improves surival of critically ischemic porcine flap, which may be mediated by induction of HO-1 and i-NOS Contaldo C, Erni D. EURAPS (Marseilles, 26.05.-28.05.2005)
- EPO administration protects critically reperfused experimental osteomycu taneous flap tissue. Contaldo C, Wanner GA, Künzi W Schweizerische Gesellschaft für Plastische und Rekonstruktive und Aesthetische Chirurgie (Biel am 30.9.05-01.10.2005)

Collaborations:

- Prof. Dr. MD Menger, Institut für Klinisch-Experimentelle Chirurgie, Universitätsklinikum, Homburg/Saar, Deutschland

Selected references:

- Plock J, Contaldo C, von Ludinghausen M. Levator palpebrae superioris muscle in human fetuses: anatomical findings and their clinical relevance. Clin Anat. 2005
- Plock JA, Contaldo C, Erni D. Is hemoglobin in hemoglobin vesicles infused for isovolemic hemodilution necessary to improve oxygenation in critically ischemic hamster skin? Am J Physiol Heart Circ Physiol. 2005
- Harder Y, Contaldo C, Erni D.Preconditioning with monophosphoryl lipid A improves survival of critically ischemic tissue. Anesth Analg. 2005

2.4 Plastic, Hand & Reconstructive Surgery Research

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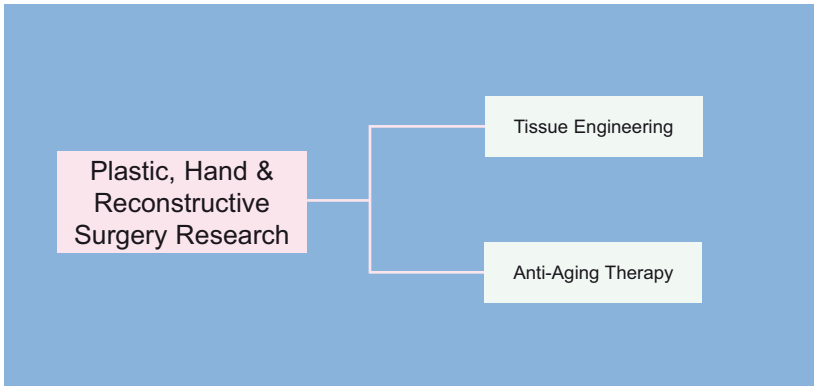
Prof. Dr. med.
Victor E. Meyer,



2.4.1 Tissue Engineering

Dr. med.
Volker Wedler

Dr. med.
Christian Köhler

Manfred Welti

The regenerative capacity of cartilage is known to be very poor and is therefore a major problem in several diseases with severe cartilage loss - such as rheumatoid arthritis - traumatic articular defects and cartilage defects secondary to trauma or tumorsurgery. The reconstruction of a destroyed joint has remained a difficult problem. The ability to reconstitute tissue structure and function in vitro has tremendous clinical implications and is likely to become very important in coming years. However, several problems remain. One factor of major importance is the quality of the engineered cartilage and the quality of the extracellular matrix in particular.

Engineering of articular cartilage using PEGT/PBT copolymer carriers and autologous grafting to repair full-thickness defects in small joints
Dr. V. Wedler, Dr. Ch. Köhler, M. Welti

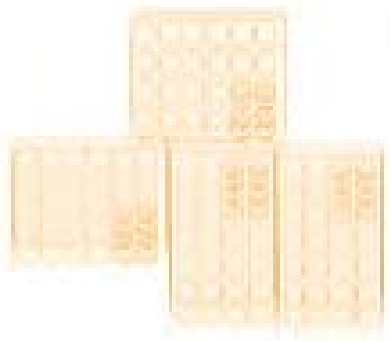
We have replaced a complete articular defect in the distal knee joint of twenty White New Zealand Rabbits with autologous chondrocytes cultured on a synthetic biodegradable scaffold. The project is a co-operation with Isotis Tissue Engineering and extends for a time period of two years. The purpose of this animal study is to determine the amount and quality of the engineered cartilage, the incorporation of the implant, degradation/replacement of the scaffold and long term biomechanical properties after 6 weeks, 3 and 6 months respectively. The implants are evaluated with CT scans, histology, DMMB assay, PCR, DNA analyse and biomechanical testing.

Collaborations:

- IsoTis Tissue Engineering, Bilthoven, Netherlands
- Department of Materials, Institute of Polymers, ETH Zurich
- Department of Materials, Polymer Technology, ETH Zurich
- Institut für Labortierkunde, Universität Zürich, Irchel

Impact of x-rays on human chondrocytes in the area of tissue engineering
Dr. V. Wedler, Dr. Ch. Köhler, M. Welti

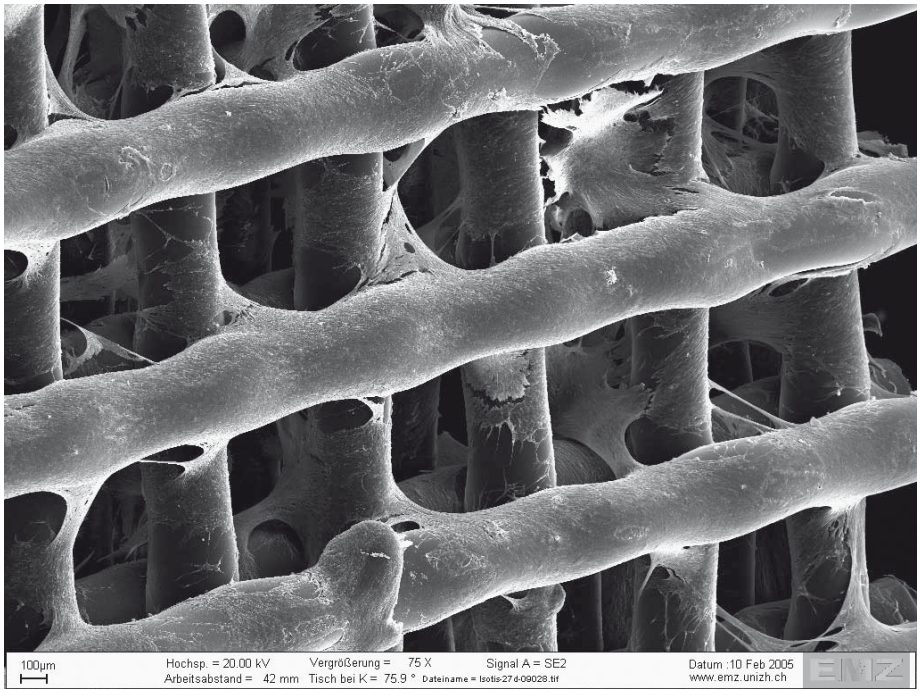
Tissue engineering of chondrocyte cultures in vitro permits the performing of analyses of the metabolism as a function of the presence and absence of different mediators. However, there are natural factors, which can have an effect on human cell cultures. The present study investigates how cartilage cells of human joints react to x-rays in quality and quantity. Special attention is paid to the transillumination check at airports. Further, this study is to contain an analysis regarding a possible calculation of the growth rate of chondrocytes by means of a mathematical formula. The test showed that after irradiation of the chondrocytes with differently large dose units, no significant differences occurred compared with the control group‘s values. This applies both to the cultures exposed to luggage transillumination as well as for those exposed to radiation devices of the department for radiology. Regarding the calculation of cell growth, a mathematical formula could be established, with which a prediction of the cell increase became possible. This corresponds to the fourth derivative of a linear function. A direct acute cell damage affecting cell increase or causing quality variances could not be observed. However, the question about long-term damage of the cell nucleus‘ DNA remains and requires further investigation. Due to our results the growth rate is predictable as a function of time.



Optical differentiation between several scaffolds in tissue engineering with human chondrocytes using light microscopy and SEM

Dr. Ch. Köhler, Dr. V. Wedler, M. Welti

At present, we are describing results in the tissue engineering of chondrocytes from human cartilages by quantitative and qualitative measurements (MTT and GAG). But some features are thereby not conclusively assessed, as for instance: design, pattern of growth or cell cohorts. In this study, we reported about various methods to show optical and photo-optical effects of four different types of scaffolds. We analysed four types of scaffolds produced in different institutes (Isotis/Netherland, Innovent/Germany and a new polymer scaffold from China). Human articular chondrocytes were isolated after traumatic amputation in the emergency room. The chondrocytes were expanded in culture flasks (either 75 cm^2) using DMEM with 10% FCS, gentamicin (50 µg/ml), and amphotericin B (Fungizone, 2.5 µg/ml). After 14, 28 and 56 days we performed a light microscopy as well as a scanning electron microscopy (SEM). Afterwards, we printed digital pictures for the measurement of growth features. The evaluation has provided us with totally new visualisation and has therefore given us new insights into the scaffold. Design and cohorts have distinctive characteristics in the different scaffold materials. In this way we are able to discover growth procedures. Light microscopy photography and SEM are appropriate means to visualise the growth and functional design of cell cultures in tissue engineering.



Preisträger BIONALE „Form follows Nature“ Wettbewerb der Deutschen Gesellschaft für Plastische Chirurgie 2005 München

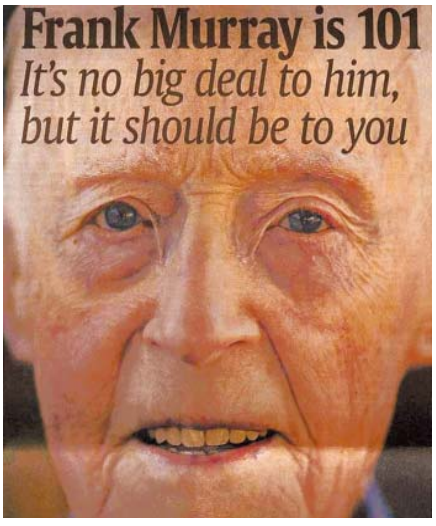
2.4.2 Anti Aging

Anti-aging therapy in tissue engineering: An approach in growing human chondrocytes
Dr. Ch. Köhler, Dr. V. Wedler, M. Welti

The advancement in medicine requires an ever-faster provision of biological tissue substitutes. This also concerns the tissue engineering of cartilage cells. In order to provide patients and physicians as fast as possible with autologous substitutes in the case of degenerative illnesses or after trauma, it is necessary to grow cells with the same quality and in the same quantity in a shortened time frame. In this study the effect of a combination preparation with antioxidants was tested with respect to an increase of the cell growth rate. We used human cartilage cells. In the process the sterile filtered and solved substance was added to the culture medium and a quantitative (MTT and GAG test) and qualitative analysis (histology) conducted over the course of ten days. The histological evaluation shows vital cells both in the vitamin and the control group. Analysed over the course of ten days in the MTT, the chondrocytes with vitamin additive reach the maximum quantity one day earlier and exceeds the total number in cartilage cells in relation to the control group. The GAG test corresponds to the results of normal chondrocyte cultures. The addition of different growth factors permits growth acceleration of cartilage cells with the same quality and quantity.

Anti Aging therapy- a new business for plastic surgeons?
Dr. Ch. Köhler, Dr. V. Wedler, M. Welti

Anti Aging therapy becomes increasingly important in many disciplines of medicine. It is an old method in a trendy position. There are some other terms such as better aging, rejuvenation or age medicine that all refer to the same concept. But who works in this new field of hormones, diets and sports therapy? There are many institutes offering their services. In this study, we analysed actual treatments in Anti Aging therapies and tried to draw a line between scientific and commercial Anti Aging procedures. We drew a comparison of actual promotions on several websites and in scientific publications on PUBMED. After that we described the main priorities of dermatology, plastic surgery, gynaecology and other disciplines. A historical search of Anti Aging therapies was analysed by several antiques books (18th and 19th century). The main business is controlled by the cosmetics industry. Plastic surgery is primarily concerned with operative treatment (Botox, Liposuction etc). Compared to this we find abundant scientific publications in all disciplines. Looked at retrospectively, Anti Aging has been established for a long time. Anti Aging therapy should only be performed by persons who know what they are doing. An interdisciplinary team is a decisive factor. It is important to know the historical facts and scientific consolidated findings.



Collaborations:

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- Prof. Dr. von Rechenberg, Tierspital Zürich
- Dr. J. Roos, Departement für Radiologie, USZ
- Dr. P. Neuenschwander, ETH Hönggerberg, Biomaterials
- Dr. M. Schnabelrauch, INNOVENT Jena
- Dr. Thomas Scholz, Departement Chirurgie USZ
- Dr. Ludwig Labler, Departement Chirurgie, USZ
- Dr. Sen, Departement of Orthopaedic Surgery. University of California, San Francisco
- Mazda Farshad, Departement Chirurgie, USZ

Selected references:

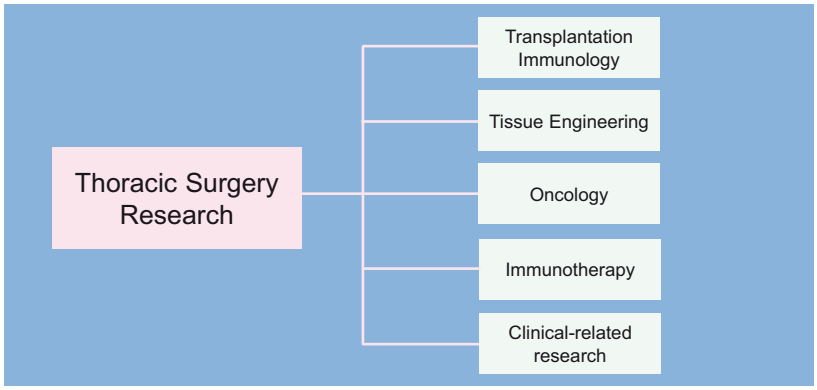
- The value of the 3D gadolinium magnetic resonance angiography (MRA) versus the conventional digital subtraction angiography (DSA) in the presurgical planning of reconstructive surgeries in the area of the lower extremities (under review)
  C. Koehler, D. Weishaupt, W. Kuenzi, V. Wedler
- Anti-aging therapy in tissue engineering: An approach to growing human chondrocytes (under review)
  C. Koehler, M. Guggenheim ,J. P. Hellermann, B. Bode, V. Wedler
- Oropharyngeale Rekonstruktion mittels freiem Jejunumtransplantat nach Tumor- und Stenosenresektion: Analyse von 53 Fällen (accepted 12/ 05 Hand- Plastische und Mikrochirurgie)
  N. Krügel, C. Koehler, V. Wedler, W. Künzi
- Vacuum assisted closure: specific indications (under review)
  C. Koehler, F.J. Jung, T. Scholz, L. Labler, A. Jandali, M. Comber, W. Kuenzi, V. Wedler
- Anti-aging therapy in tissue engineering: An approach in growing human chondrocytes (under review)
  C. Koehler, J. P. Hellermann, B. Bode, M. Welti, V. Wedler
- Iatrogenic neurovascular entrapment injuries caused by reduction and intramedullary fixation of fractures of the lower limb (accepted 12/ 2005 European Journal of Trauma)
  V. Wedler, L. Labler, Ch. Köhler, M. Guggenheim, W. Künzi, O. Trentz
- Extensive hydrofluoric acid injuries: A serious problem
  V. Wedler, M. Guggenheim, M. Moron, W. Künzi, VE Meyer
  J Trauma, 58(4):852-7, 2005
- A comparative analysis of phenotype expression in human osteoblasts from heterotopic ossification and normal bone
  Handschin AE, Egermann M, Wedler V, Trentz O, Hemmi S, Trentz OA A Langenbeck's Archives of Surgery, (accepted: 15.12.2005)

2.5 Thoracic Surgery Research


PD Dr. med.
Stephan Korom


Dr. med.
Sven Hillinger


Prof. Dr. med.
Walter Weder



2.5.1 Transplantation Immunology


PD Dr. med.
Stephan Korom


Dr. med.
Sven Hillinger


Dr. med.
Markus Cardell


Dr. med.
Ilhan Inci


Dr. med.
Wei Zhai


Dr. med.
Stephan Arni

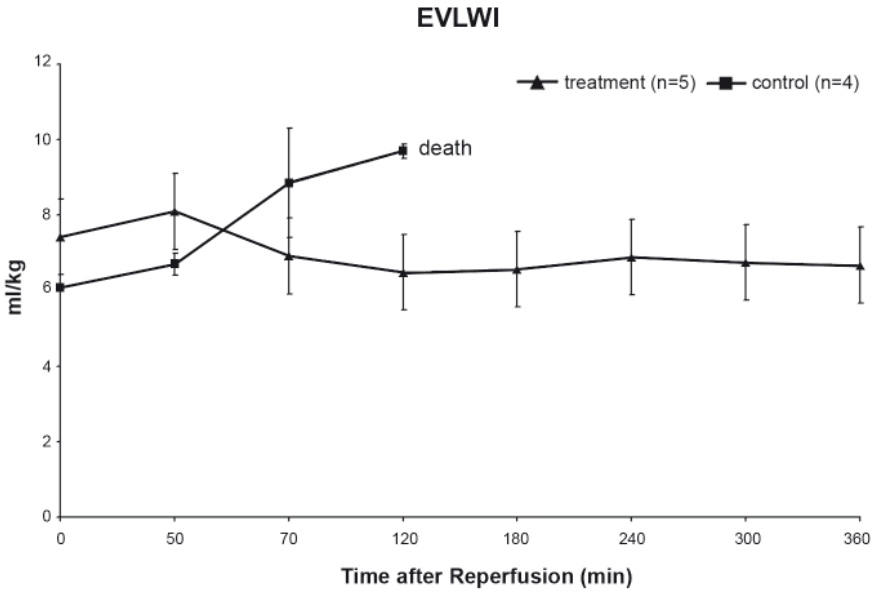

Dr. med.
Quiag Tan

Lung transplantation has become an effective therapeutic option in the treatment of patients with end-stage pulmonary diseases. However, early acute graft dysfunction continues to be a serious obstacle to successful lung transplantation, accounting for significant postoperative morbidity and mortality. In our established large and small animal models of unilateral lung transplantation we investigated different substances in terms of early graft function improvement.

Sildenafil extends Survival and Graft Function in a large Animal Lung Transplantation Model
S.Hillinger, M.Cardell, W.Zhai, Q.Tan, S.Korom

Restoring intracellular cGMP and inducing NO-synthesis attenuates ischemia-associated early pulmonary allograft dysfunction. Phosphodiesterase-5 (PDE), predominantly expressed in lung tissue, plays a pivotal role in modulating the cGMP/NO-synthase pathway in endothelial and epithelial cells. In this study we evaluate the effect of employing sildenafil (Viagra®), a specific inhibitor of PDE-5, to counteract ischemia/reperfusion (I/R) injury in a single lung transplantation model of extended ischemia. Donor animals (weight matched outbred pigs, 28-35kg) in the treatment group (I) (n=5) were injected with 0.7mg sildenafil/kg into the pulmonary artery (PA) prior to inflow occlusion. For perfusion, Perfadex®, containing 0.7mg sildenafil/l was used, and the graft stored at 1 ºC in the perfusion solution. After 24 hour ischemia, unilateral left lung transplantation was performed. Starting at reperfusion, group I received continous sildenafil (0.7mg sildenafil/kg), over six hours. Except for the sildenafil application, the control group (II) (n=4) was treated identically (PGE_1 was injected into the PA). One hour after reperfusion, the right main bronchus (MB) and right PA were occluded. Over the next five hours, cardiopulmonary parameters (systemic aterial, PA, central venous, left atrial pressure, pCO_2, pO_2) were measured, including extravascular lung water (EVLW). Thiobarbituric acid-reactive substance assay (TBARS) and myeloperoxidase (MPO) analysis from lung tissue were run. All recipients of group I survived the six hour reperfusion period, in contrast, all control animals died within 1-2 hours after occlusion of the right side.

In comparison to a marked rise in pulmonary vascular resistance (PVR) in group II (>1000 dynes/sec/cm-5), PVR in group I remained stable, moderately elevated from baseline (baseline: 150-180 dynes/sec/cm-5 vs. endpoint: 1000 dynes/sec/cm-5). EVLW in group I did not increase during reperfusion (baseline: 6.75 ± 1.4mg/kg vs. endpoint: 6.7 ± 1.0mg/kg), in contrast to group II, where pulmonary edema at two hours reperfusion preceded terminal graft failure (group I: 6.48 ± 1.8mg/kg vs. group II: 9.7 ± 0.1mg/kg). Tissue reactive free radicals at endpoint measurement in group I did not differ significantly from native tissue, yet, when compared to specimen taken from group II at time of terminal graft failure, a significant increase in free radicals was noted (group I: 13.8 ± 1.6 pmol/g vs. group II: 18.5 ± 3.0 pmol/g, $p < 0.05$).
Sildenafil treatment prevents terminal early graft failure, allowing lung transplantation after 24 hours ischemia time. Reperfusion edema was strikingly diminished, preserving pulmonary structural integrity and functional while prolonging graft ischemia time. Employing the established PDE-5 inhibitor sildenafil during lung perfusion, storage and implantation, ischemic tolerance may be extended and early graft function improved.



Circadian Melatonin Secretion During Alloantigen Challenge and Immunosuppression

M.Cardell, W.Zhai, FJ.Jung, S.Hillinger, S.Korom

Melatonin (MLT) displays a dose-dependent immunoregulatory effect, where high-dose therapy has been shown to abrogate acute rejection and significantly prolong graft survival. Endogenous melatonin secretion, in response to heterotopic rat cardiac allograft transplantation, was investigated during the acute rejection phase and under standardized immunosuppessive maintainance therapy with CsA and rapamycin.

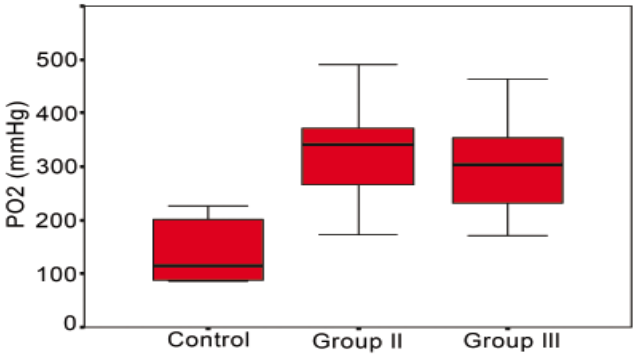
Cardiac grafts were harvested and transplanted to the abdominal great vessels. Recipients (n=6/groups) of syngeneic transplants (LEW to LEW, group I), untreated recipients of allogeneic grafts (LBNF1 to LEW, II), and allografted, CsA- (III) or rapamycin-treated (IV) hosts constituted the experimental groups. Endogenous circadian melatonin secretion was measured with a novel species specific RIA for MLT, without alteration of the light/dark cycle in recipients following transplantation, and correlated with the ensuing acute rejection response. Furthermore, the influence of therapeutical immunosuppressive regimens with CsA (2,5mg/kg bw) and rapamycin (3mg/kg bw) on circulating melatonin levels over time was analyzed.

Neither the operative procedure alone, nor the allogeneic challenge with a perfused allograft undergoing acute rejection influenced the endogenous melatonin secretion pattern could. Whereas immunosuppression by CsA did not affect circulating melatoni kinetics, rapamycin therapy significantly lowered the circadian secretory amplitude of endogenous melatonin.

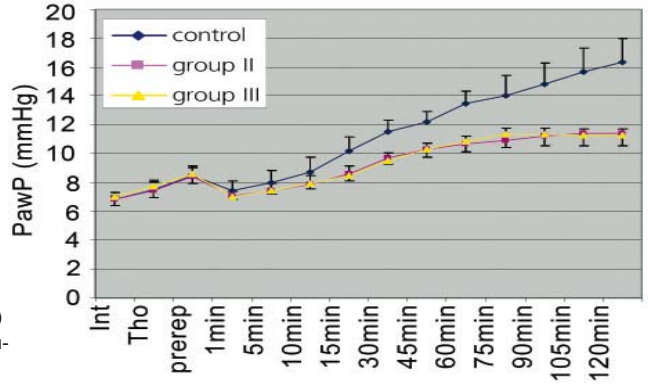
Taken together, endogenous melatonin secretion is a stable mechanism. The natural course of circadian endogenous melatonin secretion displays a robust mechanism, which is unaltered by perfused allograft transplantation.

CD26/Dipeptidyl-Peptidase IV (DPP IV) Inhibition Attenuates Posttransplant Pulmonary Ischemia/Reperfusion Injury after Extended Ischemia
W.Zhai, M.Cardell, I.Inci, S.Arni, S.Korom

The T cell costimulatory Ag CD26 possesses dipeptidyl peptidase IV (DPP IV) catalytic activity, which is linked to its costimulatory efficacy. Based on our observation that abrogation of acute pulmonary rejection and preservation of ventilatory function can be achieved by DPP IV inhibition in an rat allogeneic lung transplantation model, to elucidate the non-immunological, enzymatic-activity-associated effect of CD26/DPP IV following transplantation, in this study we investigated the impact of specific catalytic inhibition of CD26/DPP IV on ischemia-reperfusion (I/R) injury. A syngeneic rat orthotopic left lung transplantation model was used. Group I animals consisted of control group, donor lungs were flushed and preserved in Perfadex for 18 hours at 4 C and then transplanted and reperfused for 2 hours; Group II animals received the same procedures as group I except 25umol/L DPP IV inhibitor in both flush and storage solutions; Group III animals received the same procedures as group I except 25umol/L DPP IV inhibitor in flush solution only (6 Tx/group). After 2-hour reperfusion, blood gas analysis and peak airway pressure (PAwP) were measured, lung tissue biopsy specimens were obtained for assessment of wet/dry weight ratio, myeloperoxidase activity (MPO) and thiobarbituric acid reactive substances (TBARS).In comparison with control group, two DPP IV inhibitor treated groups significantly improved postreperfusion graft pO2, PAwP, wet/dry weight ratio and lipid peroxidation ($p < 0.05$, respectively). In this experimental model, posttransplant lung ischemia reperfusion injury can be attenuated by selectively targeting graft DPP IV enzymatic activity.



Graft oxygenation at the end of 2h reperfusion



Peak airway pressures (PawP) in mmHg during 2-hour reperfusion period

Effect of N-Acetylcysteine on lung ischemia-reperfusion injury in rat single lung transplantation

I.Inci, S.Arni, W.Zhai, M.Cardell

Several studies have shown that agents such as prostaglandins; the oxygen free radical scavengers superoxide dysmutase, catalase, glutathione, allopurinol, dimethylthiourea, lazaroids, trimetazidine; aprotinin; platelet factor antagonists; and angiotensin converting enzyme inhibitor, captopril and melatonin to be effective in protecting lung against ischemia-reperfusion injury. N-Acetylcysteine (NAC) is a precursor of the most important physiological antioxidant glutathione. Sulphydril-containing compounds, especially reduced glutathione (GSH), are important in the protection of cells against hydroperoxide damage. This important reducing agent and antioxidant is involved in maintaining the cellular oxidation-reduction balance, and has been shown to protect cells from a wide variety of endogenous and exogenous insults. GSH can also scavenge free radicals produced by oxidative challenges.There have, therefore, been many suggestions that reduced GSH may be useful therapeutically as an antioxidant and cytoprotective agent. In this experimental study we wanted to investigate whether donor and recipient treatment with NAC would reduce ischemia-reperfusion injury following lung transplantation after 18 hours of cold ischemic storage in a rat single lung transplant model. Orthotopic single left lung transplantation was performed in male Fischer (F344) rats weighing 280-300 g, using a cuff technique for the anastomoses. Animals were randomized into two groups (n=5, each); ischemic control (IC) (group I) group; 18 hours cold (4°C) ischemia followed by transplantation, intraperitoneal saline injection (1 ml) 15 minutes before harvest and reperfusion, respectively, no treatment; NAC treated (group II) group; transplantation was carried out after 18 hours of cold ischemia (4°C), donor and recipient treatment with intraperitoneal injection of 150 mg/kg NAC 15 minutes prior to harvest and reperfusion, respectively. Right donor lungs (n=5) were assessed for GSH, MPO and TBARS in order to obtain baseline values in normal lung. Oxygenation 2 hours after graft reperfusion was higher in the NAC group (184.5±83.3 mmHg) than in the IC group (67.3 ± 16.4 mmHg) (p=0.016). Peak airway pressure (PawP) measurements made during the reperfusion period showed significant differences among the groups (p=0.015). At the end of 2-hour reperfusion PawP was 14.4±1.6 cm H2O in NAC group, and 19.2±2.2 cmH2O in IC group (p=0.008) (Fig. 1). The amount of lipid peroxidation was significantly higher in IC (17.46±10.6 micromol/g) compared to NAC (7.34±1.9 micromol/g) group (p=0.016). Reduced glutathione (GSH) level in the normal lung was 37.6±5.4 µM. GSH levels in IC and NAC groups were 6.8±0.9 µM and 20.6±2.4 µM, respectively. The difference between the groups was statistically significant (p=0.004) (Fig. 2). Myeloperoxidase activity (MPO) in IC and NAC groups were 0.52±0.44 units/g, 0.34±0.4 units/g, respectively. The difference between the groups was not significant (p=0.3). W/D weight ratio in IC was 8.2±105 and 6.7±1.6 in NAC group. The ratio was not different statistically between the two groups (p=0.2). In this model, we have shown that recipient and donor treatment with NAC protected lungs from reperfusion injury after prolonged ischemia. This effect could be due to NAC's free radical scavenging activity.

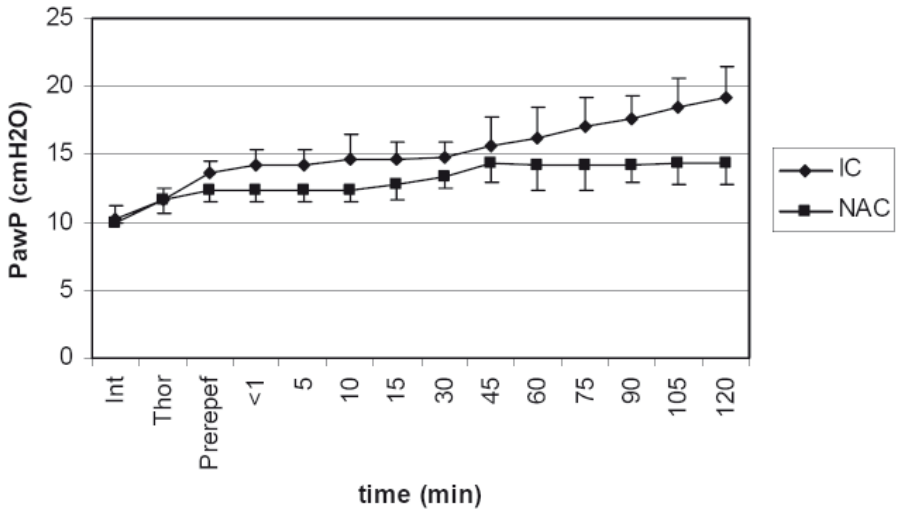


Fig 1. Peak airway pressures (PawP) in cm H2O during the 2-hour reperfusion period. The analysis of variance for repeated measures using all measurements made during the reperfusion period differed significantly between the IC and NAC groups (p=0.015). At the end of 2-hour reperfusion , PawP was significantly less in the NAC group than in the IC group (p=0.008). (Int= intubation; Thor= when the thorax is opened; prerep = just before reperfusion)

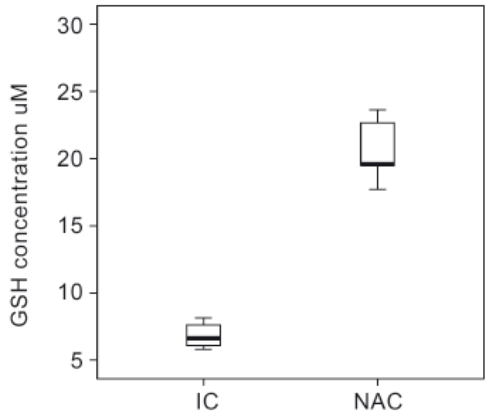


Fig. 2. Lung tissue reduced glutathione (GSH) levels between the groups showed a better preservation of GSH in NAC group compared to IC group (p=0.004).

Achievements 2005

- M.Cardell was awarded a grant for the Sildenafil project from the Hartmann-Müller foundation
- Sildenafil extends survival and graft function in a large animal lung transplantation model
  S.Korom, S.Hillinger, M.Cardell, W.Zhai, Q.Tan, W.Weder,
  EACTS/ESTS Annual Meeting, Barcelona, Spain, Sept.2005, paper in press
- Melatonin, CD26, NAC manuscripts in preparation
- M.Cardell was teaching orthotopic rat lung Tx in the microsurgery course of the Division of Surgical Research Oct. 2005

Collaborations:

- Drs. Manz und Welp, Labor Diagnostika Nord GmbH & Co KG, Nordhorn, Deutschland
- Dr. I. De Meester & Prof. Dr. S. Scharpé from the Dept. of Pharmaceutical Sciences, University of Antwerp, Antwerp, Belgium
- PD Dr. M. Keel and Dr. L. Härter, Dept. of Traumatology, USZ, Zurich

Selected references:

- F. J. Jung , L. Yang , L. Härter, I. Inci, D. Schneiter, D. Lardinois, M. Keel, W. Weder S. Korom. Melatonin in vivo prolongs cardiac allograft survival in rats. Journal of Pineal Research, 37: 36-41 (2004).
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2.5.2 Tissue Engineering

Dr. med.
Qiang Tan

Manfred Welti

Novel aspects of tissue engineered trachea
Q.Tan, M.Welti

Our research focuses on the reepithelialization of tissue engineered trachea. To achieve this goal, we have to overcome three main obstacles:

1. Identify epithelial cells source
2. Optimize epithelial cells seeding method
3. Expediate revascularization process

Regarding the source of epithelial cells, our hypothesis is that it is not the kind of epithelial cells but the intactness of the basement membrane which plays a key role in the reepithelialization of tissue engineered trachea. Many researchers around the world have proved that obtaining enough tracheal epithelial cells through autologous biopsy is very difficult. Therefore in our study we plan to use skin keratinocytes, or even skin fleet, instead of tracheal epithelial cells as the epithelial source. Fortunately, in the field of tissue engineering, tissue engineered skin remains the most successful branch so far. We plan to reconstruct tissue engineered skin first in vitro then used it to cover the inner surface of tracheal scaffold. In the former exam we have established the porcine epithelial cell culture protocol and touched upon the reconstruction of tissue engineered skin.

With regard to cell seeding process, many earlier papers suggest that the direct epithelial cell fleet seeding method works more efficiently than the cell suspension seeding alternative. Accordingly, we decide to directly adhere tissue engineered skin or fresh autologous thinner skin fleet to the scaffold with the help of stent which has been already widely used to cure benign tracheal stenosis in clinic.

Finally, revascularization remains to be the most difficult. Traditionally, tissue engineered trachea is pre-implanted into the omentum and then transplanted with pedicel blood vessels as a tracheal replacement. This, however, is proven to be a complex process that entails laparotomy twice, with high risk of complication. We therefore bring forward a novel concept of “in-vivo bioreactor” defined as the design of a perfusion system inside the scaffold.The device consists of two parts: an extracorporeal pump system and an intracorporeal perfusion tube inserted into the tissue engineered trachea scaffold. The idea is simple: the extracorporeal pumps will continuously administrate medium through the porous perfusion tube into the tissue engineered trachea to sustain the seed cells before a functional capillary net takes shape from normal tissues around. The process resembles the principle of heart-lung machines to maintain the normal blood circulation during heart operations. Through this perfusion system fresh seed cells can also be added to refresh the seed cells. This offers a clear advantage in emergency treatment when we have not enough time waiting for the cell culture. Apparently we can also deliver various growth factors to facilitate seed cells differentiation or accelerate the angiogenesis process. All the above three possibilities have already been proved in previous in vitro researches in our lab. Since we cannot accurately mimic in vitro the entire in-vivo environment, a problem constantly bothering researchers on bioreactors, it makes sense to treat the recipient himself as a bioreactor. Therefore we name the design “in-vivo bioreactor”.

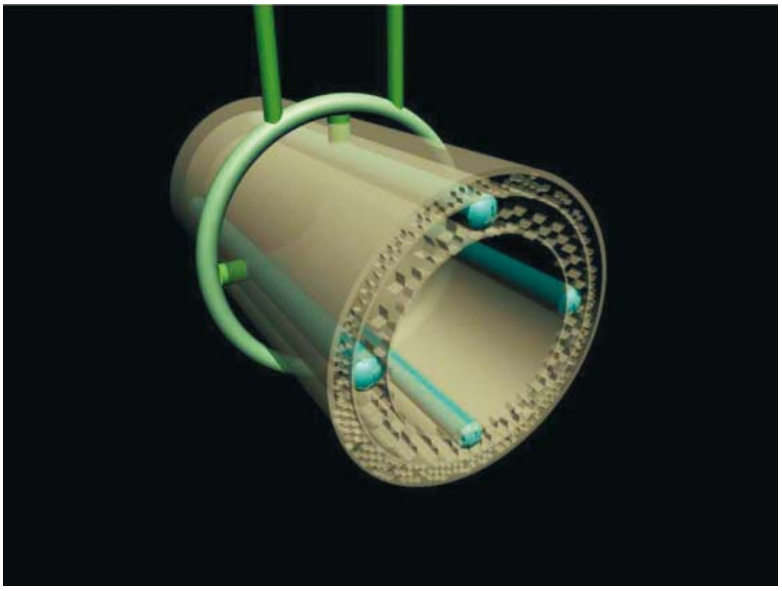


Fig. TE1. sketch of tissue engineered trachea with in-vivo bioreactor design.

A simple test model was established by inserting a porous catheter which connected to a pump system into a tubular Degrapol scaffold. Tests were performed on the advantages of maintaining the survival of epithelial cells seeded onto the DegraPol surface and of effectively delivering chondrocytes into the scaffold. Furthermore, based on chorioallantoic membrane CAM angiogenesis model, we judged its effect on the scaffold angiogenesis process. Scanning electronic microscopy SEM results show living epithelial cells after two weeks' support by in-vivo bioreactor. In cell delivery test, MTT results proved no significant difference between in-vivo bioreactor delivery group (0.312±0.177) and directly seeding static culture group (0.288±0.053). Regarding angiogenesis test, in-vivo bioreactor groups presented more tissue in-growth and capillary formation. These results demonstrated that through in-vivo bioreactor, we can deliver and maintain the survival of seeded cells as well as accelerate the scaffold angiogenesis process.

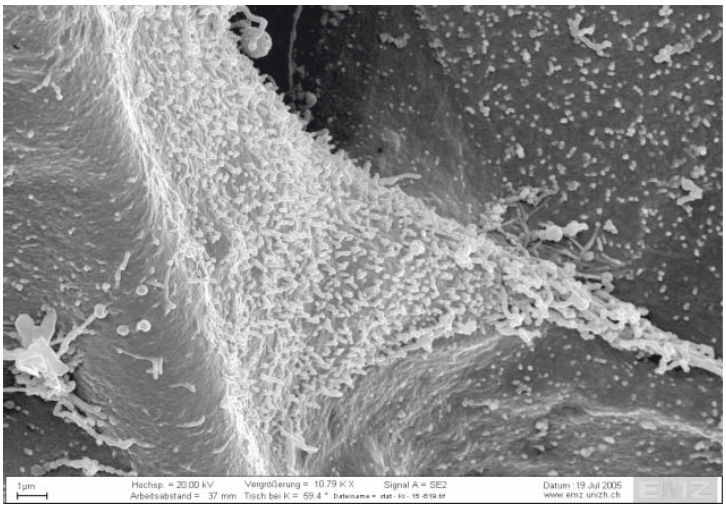
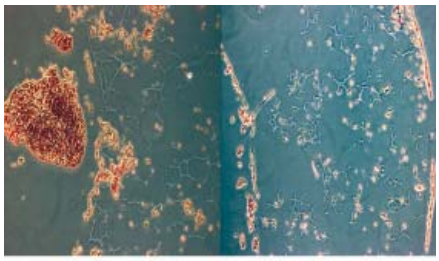
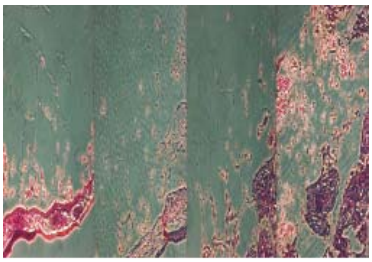


Fig TE2: SEM result show 16HBE14o cells adhered well to the DegraPol scaffold surface and survive after 2 weeks supported by in-vivo bioreactor.



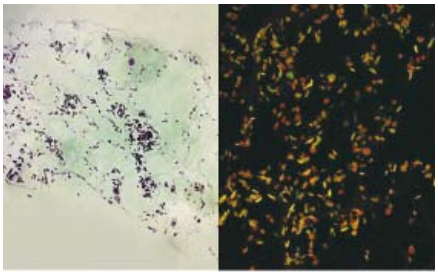
A B

Fig TE3: A: Histological slice from in-vivo bioreactor group showed larger chondrocyte cluster formation. B: In comparison, there is only a monolayer of chondrocytes covered the surface of DegraPol tube in directly seeding static culture group. (hematoxylin and eosin staining, ×100)



A B C D

Fig TE4: Scales of tissue in-growth in angiogenesis test. A: grade 0, no tissue in-grow B: grade 1, CAM tissue invades less than 1/3 part of the DegraPol tube C: grade 2, tissue invasion more than 1/3 less than 2/3 part of the DegraPol tube D: grade 3, CAM tissue invasion include the whole DegraPol tube (hematoxylin and eosin staining, ×200)



A B

Fig TE5: A: In the IV group erythrocytes migrate throughout the whole DegraPol scaffold, even at the upper part of the Degrapol tube without any other in-growth connective tissues, due to increased vessel permeability caused by VEGF. (hematoxylin and eosin staining, ×200) B: Bisbenzimide H 33342 staining marked all the erythrocyte nuclear red, proved they were migrating from normal function vessels.

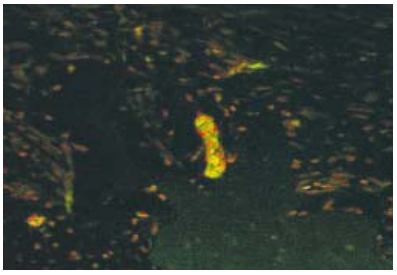


Fig TE6: Bisbenzimide H 33342 staining presents normal functional capillary formation in Degrapol scaffold in-growth tissue. The dye was injected into CAM vein far away form the DegraPol scaffold and marked the nuclear of all the cells which irrigated by normal circulation system red.

Achievements 2005

- Grant EMDO-Stiftung, manuscripts in preparation

Collaborations:

- Dr. P. Neuenschwander, Institute of Polymer Research, ETH, Zurich, Switzerland.
- Dr. L. Moroni, Twente University, IsoTis S.A., Bilthoven, The Netherlands.
- Tissue Engineering Groups, Division of Surgical Research, USZ, Zurich.
- Dr. R.Steiner, Onkologie, USZ

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  Tissue engineered cartilage generated from human trachea using DegraPol scaffold. Eur J Cardiothorac Surg. 2003 Aug; 24(2): 201-7.

2.5.3 Oncology


Dr. med.
Sven Hillinger


Dr. med.
Stephan Arni


Dr. med.
Isabelle Opitz


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Manfred Welti


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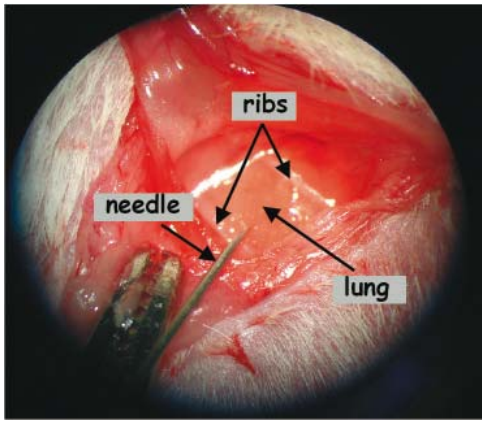
Loco-regional chemokine treatment inhibits tumor growth in an orthotopic model of lung cancer
S. Hillinger, M.Cardell, S.Arni

Effective anti-tumor responses require both antigen presenting cells and lymphocyte effectors. Although lung cancers express tumor antigens, they are ineffective as antigen presenting cells because tumor cells often have limited expression of MHC Ags and lack co-stimulatory molecules. It has been demonstrated that local and systemic administration of chemokines as stimulators of the immune response is beneficial as potent anti-cancer strategy. Epstein Barr virus-induced molecule 1 ligand chemokine (ELC/CCL19) is a CC chemokine that strongly chemoattracts both dendritic cells (DC) and T lymphocytes. In this study we evaluated the anti-tumor efficacy of loco-regional ELC/CCL19 administration in an orthotopic model of bronchogenic carcinoma.
$5x10^4$ L1C2 and 3LL cells have been injected into the right upper pulmonary lobe of Balb/C and C57/Bl6 mice respectively. Two days after injection mice have been treated with right intraaxillar (lymph node region) injection of recombinant ELC/CCL19 (0.5μg/dose) three times per week for 2 weeks. For the evaluation of ELC/CCL19 mediated loco-regional anti-tumor responses, lungs were harvested three weeks after treatment and lung blocks as well as axillary lymph nodes were assessed for H&E staining to evaluate the tumor burden and analyses of tumor infiltrating T cell subsets by flow cytometry. Tumor bearing lungs were evaluated for the production of IL-10, IL-12, GM-CSF, IFNγ, TGFβ, by ELISA and PGE2 by enzyme immunoassay (EIA) in the supernatants after an overnight culture.
Histological evaluation of tumor sections and axillary lymph nodes revealed extensive lymphocytic infiltration with a marked reduction in tumor growth compared to diluent controls. Flow cytometric analysis showed a significant increase in both CD4 and CD8 subsets as well as dendritic cells. However, there was a decrease in CD4+CD25+ T regulatory cells in the tumor infiltrating lymphocytes of ELC/CCL19 treated mice lungs. Lung tissue cytokine profiles showed a shift towards immunostimulatory molecules.
These results in a clinically relevant orthotopic model of lung cancer confirm the importance of chemokines in the development of an effective anticancer-immunotherapy. Further studies are warranted to delineate the mechanisms responsible for the anti-tumor responses following ELC/CCL19 therapy for lung cancer.

Under general anesthesia a small skin incision and muscle cut is performed. $5x10^4$ tumor cells are injected under vision of the moving lung into the right upper pulmonary lobe. Inzision is closed with 1-2 single sutures.



Furthermore we have achieved first promising results of a combination treatment with the chemokine ELC/CCL19 and Interleukin-7, which amplifies the longevity of the tumor antigen specific T cells and NK effectors.

Achievements 2005

- Grants Zürich Cancer League, Sassella-Stiftung
- Presentations at the Annual Meeting of the AACR, Anaheim, Ca, April 2005, manuscripts submitted and in preparation, several grant applications running

Collaborations:

- Prof. S.M. Dubinett, Director of the UCLA Lung Cancer Program, and Dr. S. Sharma, Associate Research Professor, University of California Los Angeles

Selected references:

- Hillinger S, Yang SC, Zhu L, Huang M, Duckett R, Atianzar K, Batra RK, Strieter RM, Dubinett SM, Sharma S. Epstein Barr Virus-Induced Molecule 1 Ligand Chemokine (ELC/CCL19) Promotes IFNg-dependent Antitumor Responses in a Lung Cancer Model. J Immunol. Dec 15;171 (12):6457-65 (2003).
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Malignant pleural mesothelioma – investigation of different cytotoxic substances

I.Opitz, S.Hillinger, D.Lardinois, S.Arni

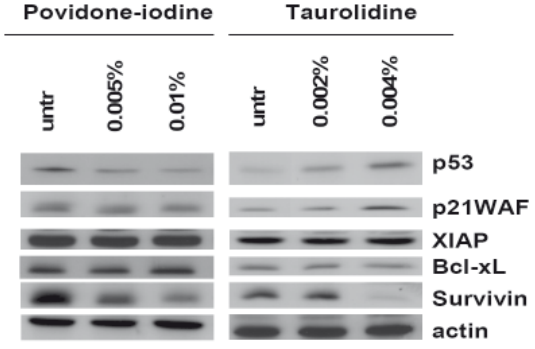
Objective: To evaluate the cytotoxicity induced by taurolidine and povidone-iodine (PVP-I) in malignant mesothelioma cells.

Materials and Methods: MTT cell viability tests were performed on four human mesothelioma cell lines (ZL 5, ZL55, ZL34, NCI-H28) and on human fibroblasts after exposure to taurolidine and PVP-I. Caspase 3-like assays, flow cytometry with Annexin V and 7AAD, and Hoechst nuclear staining were performed to assess the type of cell death induced by these agents. The effect of taurolidine or povidone-iodine on p53 activation was measured by Western blotting and their ability to sensitize cells to cisplatin was determined in MTT- and caspase assays.

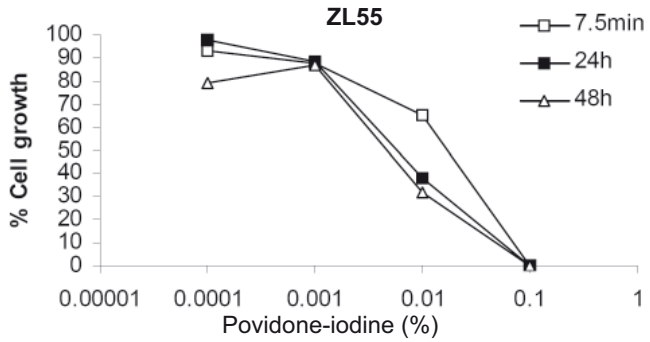
Results: Both substances killed mesothelioma cells already after 7.5 min incubation, but taurolidine was more specific towards tumour cells. Taurolidine but not PVP-I induced caspase-3-like activity in mesothelioma cell lines. Cell growth inhibition by taurolidine could be reduced by the caspase inhibitor zVADfmk. Annexin V/7AAD double labeling and Hoechst nuclear staining revealed that taurolidine induced both apoptosis and necrosis after 24 h, whereas PVP-I induced uniquely necrosis. Taurolidine activated p53 in mesothelioma cells and sensitized them to cisplatin-induced apoptosis whereas povidone-iodine had no such effect.

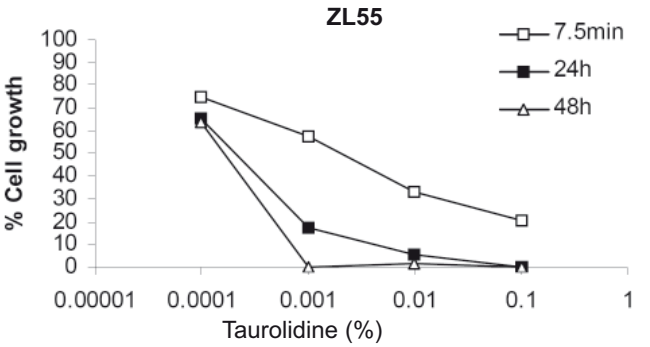
Conclusion: Both substances are cytotoxic to human malignant mesothelioma cells at early and late time points at concentrations used in clinical practice. Taurolidine induces apoptosis and necrosis in mesothelioma cells, activates p53 and sensitizes cells to cisplatin, whereas PVP-I inhibits cell growth via necrosis. Both solutions might be promising candidates for local treatment concepts for malignant pleural mesothelioma.

Furthermore we started to investigate the intrapleural use of both substances in vivo. In a first step we were able to establish the first recurrence model of malignant mesothelioma in the rat. By subpleural injection of tumour cells we observed a defined tumour growth of a reproducible size leading to local recurrence exactly at the same site after resection of the nodule. With this model we started now to investigate the influence of the intrapleural use after extrapleural pneumonectomy of taurolidine, PVP-I, CCL-19, a chemokine that influences cell mediated immune-respone at the tumour site in lung cancer therapy. Furthermore the effect of intrapleural use of cisplatin solution or cisplatin combined with a surgical sealant was observed.



Western blot after incubation of human malignant mesothelioma cell line ZL55 with taurolidine 0.002% and 0.004% and povidone-iodine 0.05% and 0.01% for 24 hours





IC50 of povidone-iodine and taurolidine after incubation of different human meosthelioma cell lines and fibroblasts

Achievements 2005

- Zürich Cancer League „New treatment approaches for local control in malignant pleural mesothelioma”

Collaborations:

- Laboratory for Molecular Oncology (Dr. phil. S. Hopkins-Donaldson and Prof. Dr. R. Stahel)
- Department of Clinical Pathology (Dr. med. P. Vogt)
- Department of Biostatistics (Dr. V. Rousson)

Selected references:

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- Docherty JG, McGregor JR, Purdie CA, Galloway DJ, O´Dwyer PJ. Efficacy of tumoricidal agents in vitro and in vivo. Br J Surg 1995; 82: 1050-1052

2.6. Surgical Intensive Care Medicine

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John F. Stover

Prof. Dr.
Reto Stocker

Silke Ludwig

Jutta Sommerfeld

The Division of Surgical Intensive Care Medicine consists of three Intensive Care Units (ICU) in which critically ill surgical and medical patients are treated. These patients suffer from various complex and complication- ridden diseases, which require intense treatment of the underlying diseases (as e.g., traumatic brain injury, pulmonary, hepatic, renal illnesses, burns) and secondary life-threatening complications (as e.g., ARDS, sepsis, multi-organ failure). Research within this field is extremely challenging as progressive pathophysiologic changes are overshadowed by evolving secondary processes and influenced by therapeutic interventions. The main aims of our research employing analytical techniques in the newly established laboratory are to 1) characterize important pathophysiologic and pharmacodynamic cascades and 2) unmask potentially unfavorable effects of routine therapeutic measures.

Our research focuses on disease- specific as well as disease- spanning changes.

A) Disease- specific changes

In regard to disease- specific changes we are investigating the impact of various brain lesions following severe traumatic brain injury on arterio- jugular venous differences in amino acids, ATP degradation products, and different cytokines which might differentiate lesion- dependent mediators from markers of cell damage and unmask a pathophysiologic important temporal profile possibly driving ensuing deterioration.

B) Disease- spanning changes

Nutritional requirements. In regard to disease- spanning changes we are investigating cellular and humoral alterations with the aims of improving nutritional requirements and reducing cellular immunodepression. This also includes assessing the influence of different enteral and parenteral nutritional solutions on potentially harmful amino acids as e.g., glutamate, arginine, and aromatic amino acids.

Effects of norepinephrine. Norepinephrine is routinely used to elevate arterial blood pressure with the aim of improving microcirculation and tissue oxygenation. However, norepinephrine may compromise this aim by excessive arteriolar vasoconstriction and activation of thrombocytes which could induce microthrombosis, thereby promoting tissue injury. In addition, norepinephrine might impair the activity of neutrophils, thereby contributing to cellular immunodepression, thus predisposing the critically ill patient to the development of infections. In this context, we have established the evaluation of sublingual *in vivo* microscopy using the novel bed side and non invasive orthogonal polarized spectral (OPS) imaging in critically ill patients which revealed a dose- dependent norepinephrine- mediated decrease in vessel diameter and functional capillary density (fig. 1).

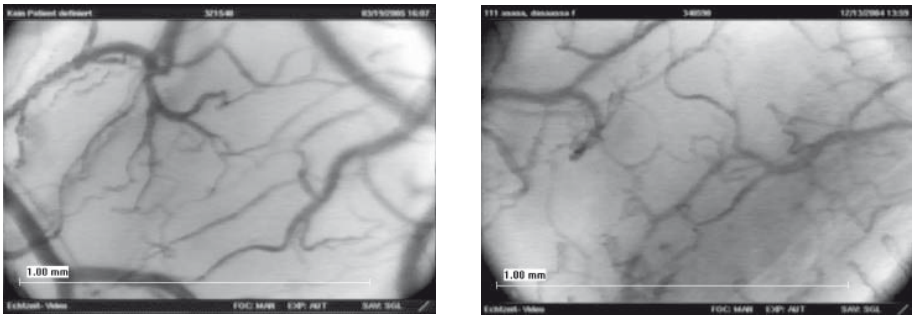

Fig. 1 Influence of norepinephrine on sublingual microcirculation determined by OPS imaging in a healthy control (left) and a patient (right) receiving high dose norepinephrine at 15 µg/ min. The functional capillary density was significantly decreased from 47.3 to 28.8 cm/ cm^2. (Doctoral thesis of Claudine Fridez)

In vitro stimulation of isolated platelets and neutrophils revealed a significant concentration- dependent increase in platelet activation (fig. 2) and neutrophil impairment (fig. 3), respectively.

Surveillance of pharmacological and metabolic coma. Depending on the underlying disease, continuous administration of analgetics and sedatives becomes indispensable. However, individual pharmacogenetics, elimination and distribution profiles might predispose the patients to iatrogenic induced drug tolerance and dependency with ensuing severe withdrawal symptoms due to cerebral transmitter system imbalance.

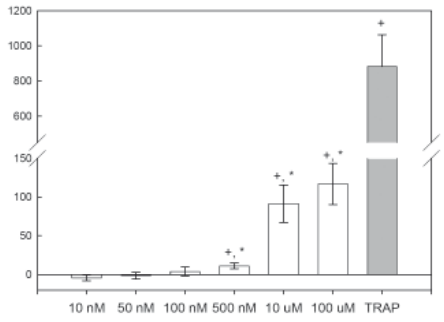

Fig. 2
Dose- dependent increase in surface P- Selectin expression in isolated thrombocytes from healthy controls stimulated by norepinephrine *in vitro (*p< 0.01 vs. low dose; +p< 0.01 vs. baseline; ANOVA)*; *TRAP*= Thrombin receptor activating protein inducing maximal stimulation. (Doctoral thesis of Christoph Tschuor)

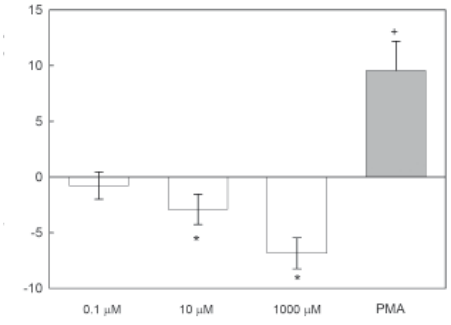

Fig. 3
Dose- dependent decrease in production of radical oxygen species (ROS) in isolated neutrophils from healthy controls stimulated by norepinephrine *in vitro* (*p< 0.01 vs. baseline; +p< 0.01 vs. norepinephrine; ANOVA); *PMA*= Phorbol 12-myristate 13-acetate (positive control). (Doctoral thesis of Martina Tanner)

Successful establishment of bed side BIS EEG in patients with severe traumatic brain injury was the first step in characterizing drug potencies of routinely applied sedatives with the aim of using a sedation depth- targeted adaptation of drug dosage, thereby possibly avoiding drug over- and underdosage. This will be complemented by analysis of specific pharmacodynamic alterations in conjunction with the analysis of drug concentrations and their active metabolites.

Achievements 2005

- Establishment of a laboratory for the Division of Surgical Intensive Care Medicine
- Approval by the local Ethics Committee
- Approval of a HPLC with an autosampler, a fluorescence and multiwave detector to perform analysis of various mediators and markers of tissue damage and determine drug concentrations
- Initiation of in house collaborations to determine changes in arterio- jugular venous differences in oxycholesterol (a marker for cerebral cholesteral degradation and membrane damage), amino acids, P-Selectin (a marker for activated thrombocytes), and changes in functional activation of isolated neutrophils and platelets
- John F. Stover was appointed executive member of the European Brain Injury Consortium (EBIC)

Collaborations:

- PD Dr. med. Marius Keel, Division of Trauma Surgery, University Hospital Zuerich
- Dr. rer. nat. Riem Ha, Department of Internal Medicine, University Hospital Zuerich
- Dr. med. Lars Asmis, Institute for Clinical Hematology, University Hospital Zuerich
- PD Dr. Guido Wanner, Division of Trauma Surgery, University Hospital Zuerich
- PD Dr. Katharina Rentsch, Institute for Clinical Chemistry, University Hospital Zuerich
- Dr. med. Oliver W. Sakowitz and Prof. Dr. Andreas W. Unterberg, Department of Neurosurgery, University Hospital Heidelberg
- Dr. med. Ulrich W. Thomale, Department of Neurosurgery, Charite, Berlin

Selected references:

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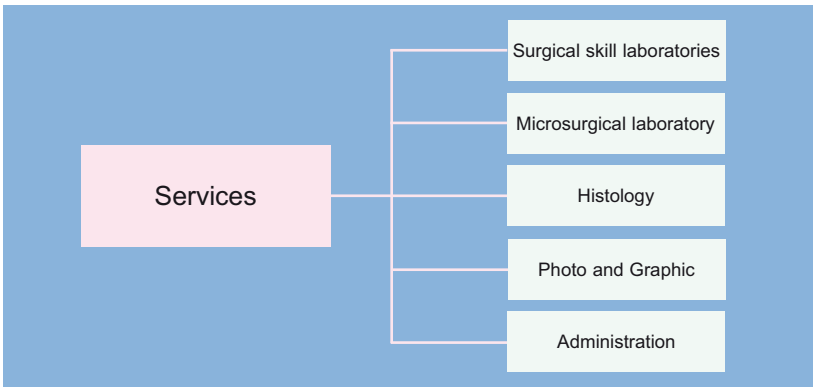
- Stover JF, Steiger P, Stocker R. Treating intracranial hypertension in patients with severe traumatic brain injury during neurointensive care. Eur J Trauma 2005; 31 (4): 308- 330

3. Services




Boris Leskosek


Alush Avdyli

3.1 Surgical skill laboratories

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


Vlasta Strohmeier

3.2 Microsurgical laboratory

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


Astrid Morger

3.3 Histology

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

3.4 Photo and graphic services

Nico Wick,
Photographer

Lea Schütz-Cohen,
Photographer

Stefan Schwyter,
Scientific
Illustrator

Carol De Simio,
Scientific
Illustrator

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- layout of printing matters
- graphic and design of illustrations for papers and books
- construction and maintainance of websites
- maintainance of the digital image archives

Susanne Frehner,
Administration
Division of Surgical
Research

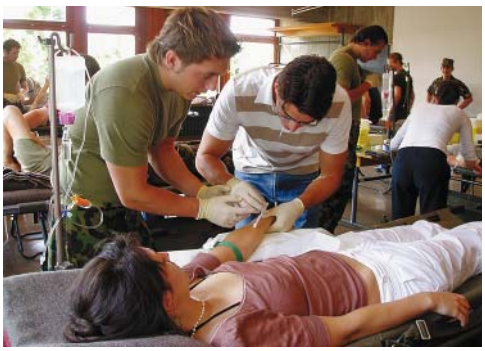
3. 5 Administration

- Administrative office management
- Financial accounting of the Research Divison
- Organisation, planning and coordination of Workshops and vocational training
- Workshop, tutorials and seminars
- Quarterly reports preparation
- Meeting coordination for the head of the Research Division
- Personnel administration of the employees of the University Hospital Zurich and the University Zurich

4. Events and Workshops at the Surgical Research Division in 2005

Newly established Virtual Skills Lab

ZKF round table



Student sewing and injection classes

Boris Leskosek's celebration of 35 years service

5. Publications 2005

- Baaijens F, Bouten C, Hoerstrup S, Mol A, Driessen N, Boerboom R. Functional tissue engineering of the aortic heart valve. Clin Hemorheol Microcirc 2005;33:197-199.
- Egermann M, Goldhahn J, Schneider E. Animal models for fracture treatment in osteoporosis. Osteoporos Int 2005;16 Suppl 2:S129-138.
- Giuliani M, Moritz W, Bodmer E, Dindo D, Kugelmeier P, Lehmann R, Gassmann M, et al. Central necrosis in isolated hypoxic human pancreatic islets: evidence for postisolation ischemia. Cell Transplant 2005;14:67-76.
- Graf R, Schiesser M, Reding T, Appenzeller P, Sun LK, Fortunato F, Perren A, et al. Exocrine Meets Endocrine: Pancreatic Stone Protein and Regenerating Protein-Two Sides of the Same Coin. J Surg Res 2005.
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- Kelm JM, Diaz Sanchez-Bustamante C, Ehler E, Hoerstrup SP, Djonov V, Ittner L, Fussenegger M. VEGF profiling and angiogenesis in human microtissues. J Biotechnol 2005;118:213-229.
- Kelm JM, Dionov V, Ittner LM, Hoerstrup SP, Fussenegger M. Design of custom-shaped vascularized tissues using microtissue spheroids as minimal building units. Tissue Engineering 2005.
- Mol A, Driessen NJ, Rutten MC, Hoerstrup SP, Bouten CV, Baaijens FP. Tissue engineering of human heart valve leaflets: a novel bioreactor for a strain-based conditioning approach. Ann Biomed Eng 2005;33:1778-1788.
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- Sharma S, Zhu L, Yang SC, Zhang L, Lin J, Hillinger S, Gardner B, et al. Cyclooxygenase 2 inhibition promotes IFN-gamma-dependent enhance ment of antitumor responses. J Immunol 2005;175:813-819.
- Sodian R, Fu P, Lueders C, Szymanski D, Fritsche C, Gutberlet M, Hoerstrup SP, et al. Tissue engineering of vascular conduits: fabrication of custom-made scaffolds using rapid prototyping techniques. Thorac Cardiovasc Surg 2005;53:144-149.

- Trentz OA, Handschin AE, Bestmann L, Hoerstrup SP, Trentz OL, Platz A. Influence of brain injury on early posttraumatic bone metabolism. Crit Care Med 2005;33:399-406.
- Zweifel M, Breu K, Matozan K, Renner E, Welle M, Schaffner T, Clavien PA. Restoration of hepatic mast cells and expression of a different mast cell protease phenotype in regenerating rat liver after 70%-hepatectomy. Immunol Cell Biol 2005;83:587-595.

6. Grants 2005

Cardiac Surgery

Grants	Title of Project	Project Leader
Federal Comission for Technology and Innovation (CTI/KTI)	In vivo evaluation of growth in tissue engineered arteries	Prof. Zünd Prof. Hoerstrup
SNF (NF46)	Cell and matrix evaluation in tissue engineering	Prof. Hoerstrup
EU Grant Framework Program 6 (BIOSYS)	Regenerative medicine in cardiovascular surgery	Prof. Hoerstrup
SYMETIS Research Grant	Cardiovascular tissue engineering	Prof. Zünd Prof. Hoerstrup
CO-ME	Robotics in cardiovascular surgery	Prof. Zünd PD Dr. Grünenfelder
BMBF	Cell preservation methods for cardiovascular surgery	Prof. Hoerstrup

Visceral & Transplant Surgery

Grants	Title of Project	Project Leader
Hepatobiliary laboratory		
SNF	Soluble mediators and cellular receptors in the ischemic liver	Prof. Clavien
SNF	Small-for-size liver transplantation: platelets and platelet-derived serotonin in the ischemic and regenerating liver	Prof. Clavien
Bonizzi-Theler	Die Schutzmechanismen in der ischämisch-präkonditionierten Leber	Dr. Rüdiger/ Prof. Clavien
Zürcher Krebsliga	Pfortaderligatur	Prof. Clavien
UBS	Role of Non-Parenchymal Cells for the Induction of Regeneration in the Steatotic Liver.	PD Dr. Selzner
Norvartis Stiftung für Medizinisch-Biologische Forschung	Hypothermic oxygenated perfusion extracorporeal of the rat liver in non heart beating donors after cold storage	PD Dr. Ph. Dutkowski
Pancreatitis laboratory		
SNF	Pancreatic Thread Proteins - Key Factors of a Sealing System for Epithelial Lesions in Pancreatic Ducts	PD Dr. Graf
Waring	Analysis of inflammatory suppression in a rat model (WBN/Kob) of chronic pancreatitis	PD Dr. Graf
Waring	Investigation of acute and chronic pancreatitis	PD Dr. Bimmler
Helen Biber Fonds	Suppression of inflammation in an animal model of chronic pancreatitis (WBN/Kob rat)	PD Dr. R. Graf
Islet-Transplantation laboratory		
SNF	Ischemic preconditioning and gene therapeutic strategies to improve islet cell engraftment in human pancreatic islet transplantation.	PD Dr. Weber/Dr. Moritz
Hermann-Klaus	Einfluss der Hypoxie auf den Apoptotischen Zelltod im Rahmen der Inseltransplantation	PD Dr. Weber/Dr. Moritz
Olga Mayenfisch	Präkonditionierung	PD Dr. Weber/Dr. Moritz
Hartmann Müller	Die Verwendung eines natürlich vorkommenden anti-mikrobiellen Peptids zur Verbesserung des Engraftments bei intrahepatischer Inselzelltransplantation	PD Dr. Weber/Dr. Moritz

Trauma Surgery

Grants	Title of Project	Project Leader
SNF	Wound Healing in Vacuum Assisted Closure-Treated Patients after Trauma: Implications of Neutrophil Activation for Accelerated Angiogenesis	Dr. Keel, Dr. Härter, Dr. Labler
Heuberg Stiftung	Modulation der Apoptose durch MAP-Kinasen in neutrophilen Granulozyten: Bedeutung in der Pathogenese der systemischen Entzündungsreaktion (SIRS) nach Trauma	Dr.Keel, Dr. Härter
AO Research Foundation	Assessment of soft tissue and periosteal microcirculation in severely open fractures using orthogonal polarozation spectral imaging	Dr. Wanner
Stiftung für wissenschaftl. Forschung der Universität Zürich	Nichterythroide Wirkungen von humanem rekombinaten Erythro-poietin in der Traumatologie und rekonstruktiven Chirurgie der Extremitäten	Dr. Wanner
Novartis AO Research Commission Hartmann Müller-Stiftung Sanofi Synthelabo	Modulation of Host Responses to Bacterial Endotoxin	PD Dr. Heinzelmann

Plastic Hand & Reconstructive Surgery

Grants	Title of Project	Project Leader
SUVA und Jubiläumsstiftung	Tissue Engineering	Dr. Wedler
Swiss Life	Tissue Engineering	Dr. Wedler

Thoracic Surgery

Grants	Title of Project	Project Leader
Krebsliga	Pharmacokinetic Study after Intrapleural Topical Application of Chemotherapeutic Agent for malignant Pleuramesothelioma in Immune Competent Rat Model	PD Dr. Lardinois
Hartmann Müller-Stiftung	Pharmacokinetic Study after Intrapleural Topical Application of Chemotherapeutic Agent for malignant Pleuramesothelioma in Immune Competent Rat Model	PD Dr. Lardinois
Hartmann-Müller-Stiftung	Sildenafil verlängert das Graft Überleben in einem Grosstier-Transplantations Modell	Dr. Cardell
Olga Mayenfisch - Melatonin (MLT)	Melatonin in der Transplantationsimmunologie	PD Dr. Korom
Krebsliga Zürich	Adjuvante intrapleurale Spüllösung nach Pleuropneumonektomie beim malignen Pleuramesotheliom	Dr. Schmitt-Opitz
Krebsliga	Epstein Barr virus-induced molecule 1 ligand chemokine in lung cancer therapy	Dr. Hillinger
Sassella-Stiftung	Epstein Barr virus-induced molecule 1 ligand chemokine in lung cancer therapy	Dr. Hillinger
NSAID Study	Assessment of the degree of pleurodesis after pleural mechanical abrasion and administration of COX-2 selective inhibitors and nitric oxide - relaesing NSAI-drugs in comparison to classical NSAIDs in a pig model	PD Dr. Lardinois
Hartmann Müller-Stiftung	Immunsuppressive und zytostatische Wirkung von Gemzitabine in tumorinokkulierten Empfängern perfundierter Organtransplanatate	Dr. Jung
SNF	The T cell costimulatory antigen CD26/Dipeptidyl Peptidase IV (DPP IV) in acute rejection of lung allografts	PD Dr. Korom
SNF	Experimental tracheal reconstruction using different developed extent tissue-engineered cylinder construct	Dr. Yang/ Prof. Weder
EMDO-Stiftung	Entwicklung eines in-vivo-Bioreaktors zur Reepithelialisierung einer Tissue-engineerten Neo-Trachea	Dr. Hillinger

7. Awards 2005

- Pfizer Forschungs-Preis.Dave H. Minimal invasive Chirurgie für die Behebung von angeborenen Herzfehler. Stiftung Pfizer Forschungs-Preis, Zürich.

- Research Posterpreis, Universitätskinderklinik Zürich, Kadner A, Dave H, Dodge-Khatami A, Balmer C, Bürki C, Bauersfeld U, Prêtre R. “Right axillary incision: a cosmetically superior approach to repair a wide range of cardiac defects”.

- Preis für den besten Vortrag: EVAR to reduce morbidity and mortality in treatment of ruptured abdominal aortic aneurysms: a seven year experience with bifurcated stent-grafts. 37. Jahrestagung der Österreichischen Gesellschaft für Gefässchirurgie, Krems, Österreich, 13.-15. Oktober 2005.

- International Society for Vascular Surgery Award 2005: Vacuum assisted closure system with direct contact to native arteries and/or vascular grafts to improve the outcome of perivascular infection. 32nd Annual Vascular and Endovascular Issues, Techniques and Horizons (VEITH) Symposium, N.Y., USA, 17.-20. November 2005.

- Best Oral Presentation, Dörthe Schmidt, 4th Clinical Day of Research, Center for Clinical Research, University Hospital Zürich

- Hartmann Mülller Stiftung, Tissue engineering using prenatal progenitors, Schmidt, D (2005)

- Auszeichnung durch die Deutschen Gesellschaft f. Herz- Thorax – und Gefässchirurgie für die Implementierung von CIRS (Critical Incidence Reporting System)

- Ph. Dutkowski, Research award from the Swiss Society of Surgery

- H. Heinrich, Research award from the Swiss Society of Surgery

- D. Dindo, Research award from the Swiss Society of Surgery

- PD Dr. Tian received a Poster Prize at 4th Clinical Day of Research, Center for Clinical Research, University Hospital Zürich

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