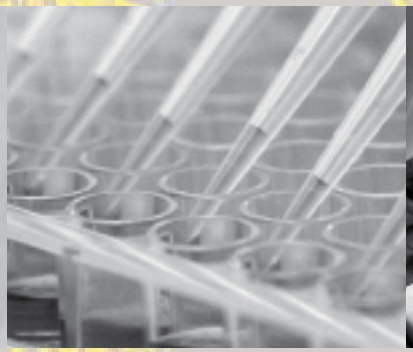
Division of Surgical Research

Annual Report 2004

Department of Surgery
University Hospital Zurich
Switzerland




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

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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 a pleasure for me to present you the Annual Report 2004 of the Division of Surgical Research, Department of Surgery at the University Hospital Zurich.

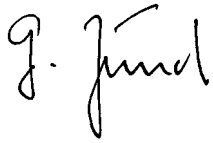
Last year we had undergone some constructions at our Division, providing us with new facilities. For the financial support we thank both the University Hospital and the University of Zurich. Especially in collaboration with the University Hospital planning team, new experimental surgery facilities to the state of the art have been created.
Due to the new lay out of the Division of Surgical Research, it was possible to extend the research working space for the Visceral-, Thorax- and Cardiac-Surgery Research groups.

The Division's quarterly internal interim reports covering the corresponding operational expenditures as well the investments carried out allow for a quality control by means of timely financial project management. The investments carried out in the past year apply to the acquisition of "Intravital-Microscope" as well " Flourescense-ELISA Readers".
Both acquisitions are being used by various research groups from our Division and therefore complementing our apparatus pool.

The weekly lectures as programmed by the University of Zürich, which are being conducted by the Division of Surgical Research at the University Hospital Zurich are regularly attended to and therefore serves as an integration within the University Hospital, University and ETH of Zürich.

It is my pleasure to thank all the employees and research partners of the University Hospital, University and ETH of Zürich 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.
Marko Turina,
Director of
Cardiac Surgery

PD Dr. med.
Michele Genoni
Director a.i. of
Cardiac Surgery

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

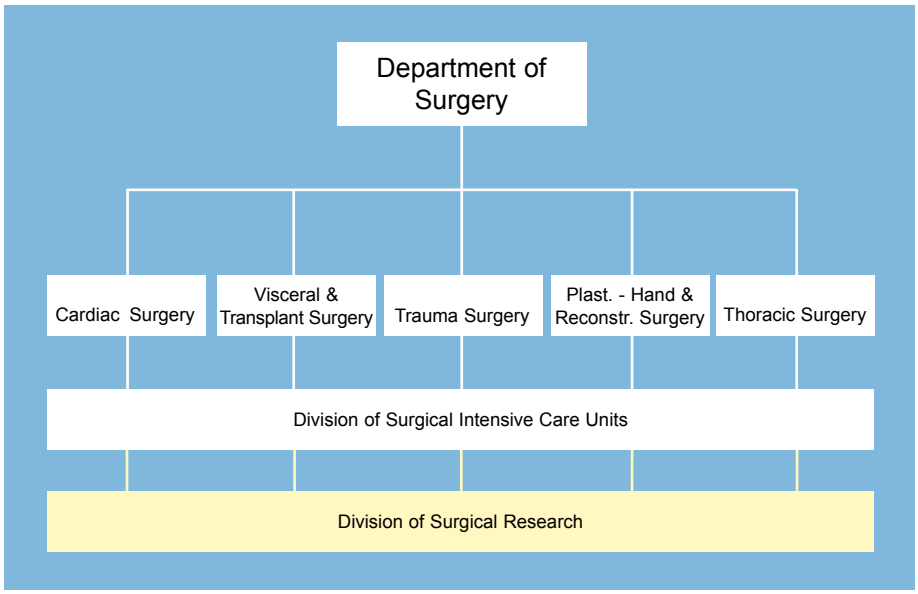
Prof. Dr. med.
Otmar Trentz,
Director of
Trauma Surgery

Prof. Dr. med.
Viktor Meyer,
Director of
Plast. - Hand &
Reconstr. Surgery

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

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

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

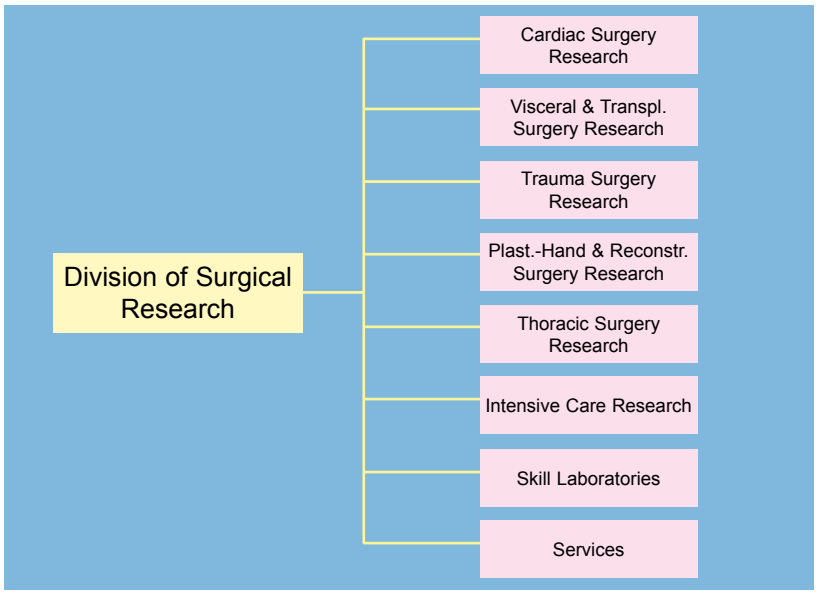


1.2 Structural Organisation of the Division of Surgical Research

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

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

Gabriella Muolo-
Giaquinta,
Administration
Division of Surgical
Research



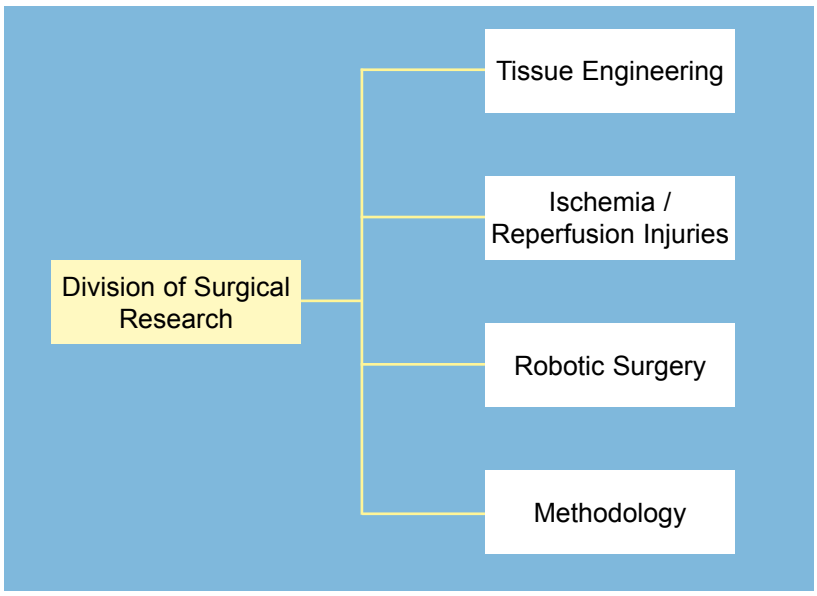
1.3 Scientific Sections within the Division of Surgical Research

Prof. Dr. med.
Simon Philipp
Hoerstrup
Tissue
Engineering

Dr. phil II
Wolfgang Moritz
Ischemia /
Reperfusion
Injuries

PD Dr. med.
Guido Wanner,
Robotic Surgery

PD Dr. phil II
Rolf Graf
Methodology



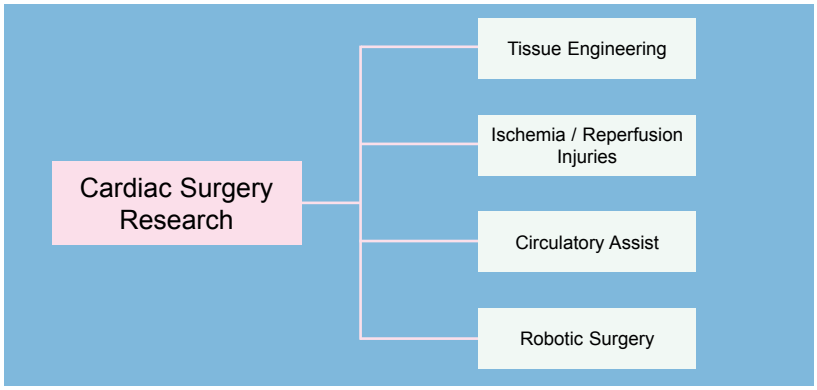
2. Research and Development

2.1 Cardiac Surgery Research


Prof. Dr. med.
Gregor Zünd


Prof. Dr. med.
Marko Turina


PD Dr. med.
Michele Genoni




Prof. Dr. med.
Simon Philipp
Hoerstrup


PD Dr. sc. nat.
Stefan
Neuenschwander


Dr. med.
Dörthe Schmidt


Dr. med.
Ian Cummings


Dr. med.
Alberto Weber


Anita Mol
PhD Student


Sirpa Price


cand. med.
Sandro Imbach


cand. med.
Armin Zürcher


cand. med.
Silvan Holdener

2.1.1 Tissue Engineering and Cell Transplantation -Regenerative Medicine

Prof. Dr. med. Simon Philipp Hoerstrup
(Director, Tissue Engineering Division; Co-Director, Cardiovascular Research)

The Laboratory for Tissue Engineering and Cell Transplantation is focused on the development and in vitro generation of novel, cell based implants for cardiovascular applications, such as blood vessels, heart valves and myocardium. Presently utilized replacements carry disadvantages for the patients mainly because non-living, artificial devices are inserted into the human organism. The technology of tissue engineering enables the in vitro production of autologous, living and functional replacements as an alternative to state of the art artificial replacements. A specific focus is the development of living devices with the capacity of growth for congenital applications.

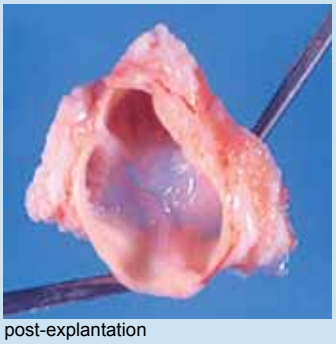
Research projects:

- Human Cell Systems (progenitor, foetal, adult)
- Extracellular Matrix (proteins, tensegrity)
- Biomaterials (biodegradable, bioactive)
- Bioreactor Systems (tension, flow)
- Biomechanics, Computational Models, Molecular Imaging
- Animal Models (small and large)
- Tissue Engineered Cardiovascular Structures (Heart Valves, Vascular Grafts, Myocardium)
- Cell Transplantation

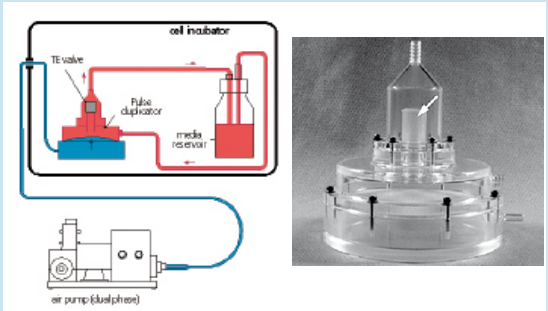
Proof of Principle Heart Valve Tissue Engineering

pre-implantation

in-vivo



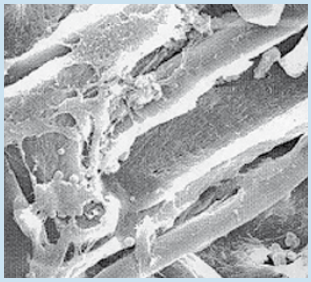
post-explantation



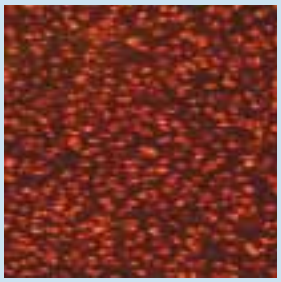
Pulse Duplicator (biomimetic)

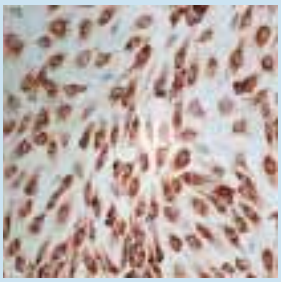
Autologous living tissue engineered heart valve in a sheep model, based on vascular derivedmyofibro-blasts and endothelial cells *(Hoerstrup et al, Circulation 2000)*

Human Progenitor Cells for Tissue Engineering of Vascular Grafts



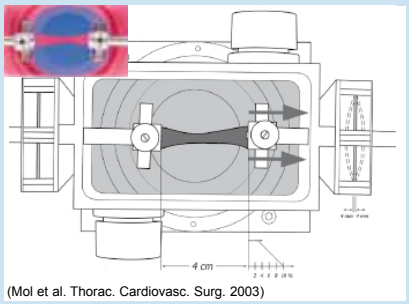
Biodegradable polymer for Tissue Engineering of vascular grafts
Scanning electron microscopy shows cell-to-polymer attachment
(Schmidt et al. STS 2004)



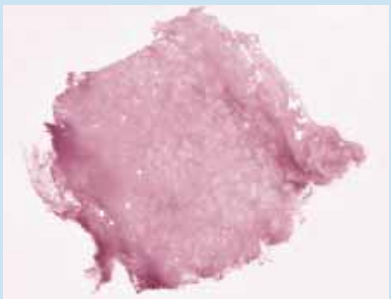


Neo-Endothelia formed by human umbilical cord blood derived endothelial progenitor cells on biodegradable vascular grafts 7 days after conditioning in a biomimetic system
(Schmidt et al. Ann Thorac Surg. 2004)

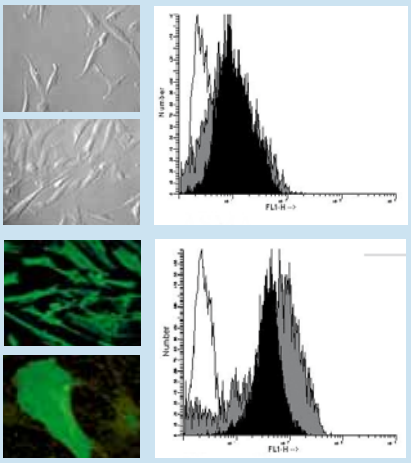
Pediatric Cardiovascular Tissue Engineering



(Mol et al. Thorac. Cardiovasc. Surg. 2003)



Living patches engineered from human umbilical cord derived fibroblasts and human umbilical cord blood derived EPCs *(Schmidt et al. EJCTS, in press)*



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

Achievements 2004

- S.P. Hoerstrup, BioSys Program, EU Framework Program 6
- G. Zünd, S.P. Hoerstrup: Pioneer Award 2004, Zürich State Bank, Switzerland
- D. Schmidt, S.P. Hoerstrup: Young Investigator Award/ Marko Turina Award, European Association of Cardiovascular Surgery
- S.P. Hoerstrup, Part time Professorship, Department of Biomedical Engineering, Technical University Eindhoven, The Netherlands

Collaborations

- 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 Biomedical Engineering, Technical University Eindhoven, The Netherlands
- 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 Biotechnology, Federal Institute of Technology, Zürich, Switzerland
- Feto-maternal Hematology Research, Department of Obstetrics, University Hospital Zürich, Switzerland
- Center for Integrative Human Physiology, University of Zurich

Selected references:

- Schmidt D, Breymann C, Weber A, Guenter CI, Neuenschwander S, Zund G, Turina M, Hoerstrup SP. Umbilical cord blood derived endothelial pro genitor cells for tissue engineering of vascular grafts. Ann Thorac Surg. 2004 Dec;78(6): 2094-8
- 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
- Hoerstrup SP, Kadner A, Breymann C, Maurus CF, Guenter CI, Sodian R, Visjager JF, Zund G, Turina M (2002) Living, autologous pulmonary artery conduits tissue engineered from human umbilical cord cells. Ann Thorac Surg,74; 46-52
- Hoerstrup SP, Zund G, Sodian R, Schnell AM, Grunenfelder J, Turina M (2001) Tissue engineering of small caliber vascular grafts. Eur J Cardio thorac Surg 20(1); 164-169
- 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 JP, Schoen F J, Mayer J E (2000) Functional living trileaflet heart valves grown in vitro. Circulation 102(III): 44-49

2.1.2 Ischemia / Reperfusion Injury

PD Dr. med.
Reza Tavakoli

Dr. rer. nat.
Anna Bogdanova

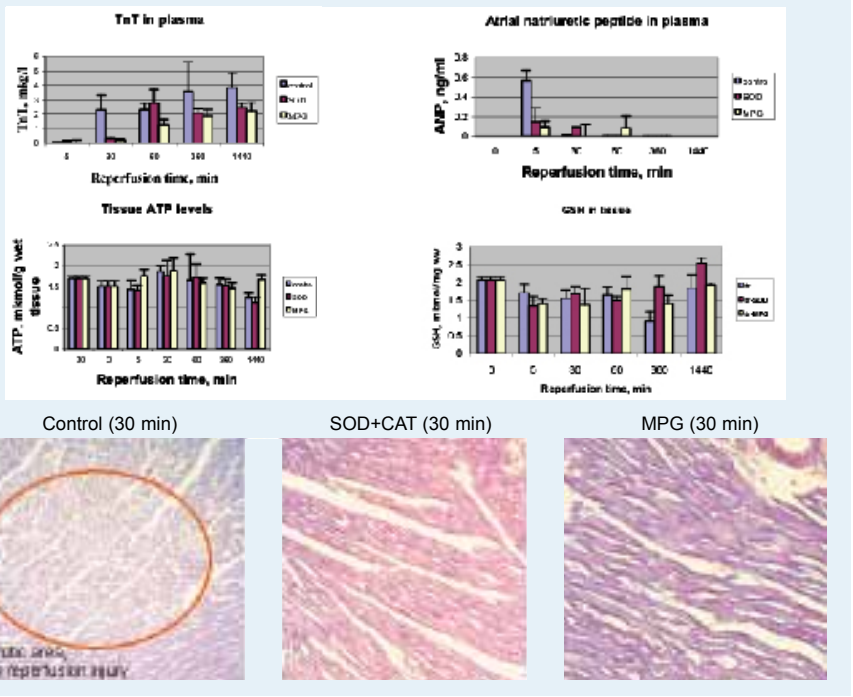
Prevention of the early wave of the myocardial ischemia-reperfusion injury using free radical scavengers

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

Objective: Oxygen-derived free radicals (ROS) are thought to be involved in the pathogenesis of tissue damage during ischemia-reperfusion injury encountered after open-heart surgery with ischemic cardiac arrest. In this study we investigate the effect of radical scavenging on myocardial reperfusion injury.

Methods: After 45 minutes of ischemia followed by 5 min, 30 min, 1, 6 or 24 hours reperfusion, heterotopic heart isografts were studied in Lewis rats. Three groups were investigated: group 1: control group without treatment, group 2 receiving dismutase+castalase (SOD+CAT) 20 min prior to reperfusion, group 3 receiving mercaptopropionyl glycine (MPG) 20 min. prior to reperfusion. Tissue metabolic and redox state was monitored using reduced and oxidized glutathione (GSH, GSSG) and ATP, myocardial injury assessed by Troponin T (TnT), atrial and brain natriuretic peptides (ANP and BNP) and inflammation by Interleukin-6 levels in plasma and myeloperoxidase activity in the heart tissue.

Results: In this model the reperfusion injury shows a biphasic pattern with an early reperfusion injury phase within 5-60 min followed by a late phase 6-24h thereafter as the inflammatory response builds up. Antioxidant treatment prevent the first wave of the post-ischemic perfusion injury, with tissue metabolic and redox state preservation and significant reduction in tissue damage and release of cardiac stress hormones (Figs). The "late" inflammatory response peaking at 6 hours is not affected by antioxidants that show poor protective effect in the late phase of ischemia-reperfusion injury.



Achievements 2004

- Oxygen-derived free radicals are involved early after reperfusion of the ischemic myocardium in development of tissue injury.
  Using ROS scavengers significantly improves post-ischemic recovery and reduces reperfusion injury.

Collaborations:

- Professor Max Gassmann, [2]Institute of Veterinary Physiology, University of Zurich
- Dr. Peter Ossent, Institute of Pathology Vetsuisse Faculty of the University of Zurich
- Dr. med. Lukas Bestmann, Institute of clinical chemistry, University Hospital Zurich

Selected references:

- Bolli, R. Myocardial ischaemia: metabolic disorders leading to cell death. Rev Port Cardiol. 1994 Sep;13(9):649-53.
- Dhalla NS, Golfman L, Takeda S, Takeda N, Nagano M.
  Evidence for the role of oxidative stress in acute ischemic heart disease: a brief review. Can J Cardiol. 1999 May;15(5):587-93.
- Boyle, JrEM, Pohlman TH, Johnson MC, Verrier ED. The systemic inflammatory response. Ann Thorac Surg 1997; 64: S31-7.
- Otani H, Engelman RM, Rousou JA, Breyer RH, Lemeshow S, Das KD. Cardiac performance during reperfusion improved by pretreatment with oxygen free-radical scavengers. J Thorac Cardiovasc Surg 1986; 91: 290-5.
- Stewart JR, Gerhardt EB, Wehr CR, Shuman T, Merrill WH, Hammon JW, Bender HW. Free radical scavengers and myocardial preservation during transplantation. Ann Thorac Surg 1986; 42: 390-3.
- Galang N, Sasaki H, Maulik N.
  Apoptotic cell death during ischemia/reperfusion and its attenuation by antioxidant therapy. Toxicology. 2000 Aug 7;148(2-3):111-8.

2.1.3 Circulatory Assist

PD Dr. med.
Mario Lachat

Dr. med.
Andreas Künzli

PD Dr. med.
Markus Wilhelm

Dr. med.
Thomas Syburra

Dr. med.
Denis
Berdajs

Assist device program
PD Dr. Mario Lachat, Dr. Andreas Künzli, PD Dr. Markus Wilhelm,
Dr. Syburra Thomas, Dr. Denis Berdajs

Bridging to Transplantation

Overall experience at USZ with implantable axial flow blood pumps (left ventricular assist device; n=22), in the treatment of terminal heart failure is very encouraging. Results of transplantation after that bridging period are excellent, as none of the patients died so far. A new generation of axial flow pump (Incor, Berlin Heart) is actually under evaluation (n=5). The innovation is that the impeller is no more mechanically suspended but levitating, due to electromagnetical forces. Reduced hemolysis is one of the major advantage of this new device (see figure 1).
Postoperative monitoring of such patients which have no palpable pulse is quite challenging. A new telemetric (GPRS based) device, Auricall (see figure 2), which was partially developed at the USZ, is able to control oxygen saturation and heart rate very accurately. Next implementation will be the integration of ECG lead, allowing to analyse rhythm disturbances.

Figure 1

Figure 2

Short-term support with the Levitronix centrifugal pump

In 2004, a total of 8 patients have been supported, for low output syndrome or respiratory failure, with the Levitronix blood pump. In several cases, support time extended above 10 days. As biocompatibility is very high, low-dosis heparinisation is sufficient, which reduces bleeding complications. There was no pump thrombosis or failure at all, and hemolysis remained very low in all patients.

Achievements 2004

Presentation on an international meeting

- S. Salzberg, M. Lachat, E. Teitel, K. von Harbou, G. Zünd, M. Turina. LVAD as bridge to Heart Transplantation in the setting of fixed pulmonary hypertension.
  Presented at the ASAIO Meeting on June 18th, 2004, Washington DC, USA

Collaborations:

- Levitronix® Inc. (Zürich, Boston, MA)
- Cardiosafe Inc (Zürich)

Selected references:

- Salzberg SP, Lachat ML, von Harbou K, Zund G, Turina MI.
  Normalization of high pulmonary vascular resistance with LVAD support in heart transplantation candidates.
  Eur J Cardiothorac Surg. 2005;27:222-5.
- Salzberg SP, Lachat ML, Zund G, Turina MI.
  Left ventricular assist device (LVAD) enables survival during 7 h of sustained ventricular fibrillation.
  Eur J Cardiothorac Surg. 2004;26:444-6.
- Salzberg S, Lachat M, Zund G, Oechslin E, Schmid ER, DeBakey M, Turina M.
  Left ventricular assist device as bridge to heart transplantation-lessons learned with the MicroMed DeBakey axial blood flow pump.
  Eur J Cardiothorac Surg. 2003;24:113-8.

2.1.4 Robotic Surgery and Innovative Technologies

PD Dr. med.
Jürg Grünenfelder

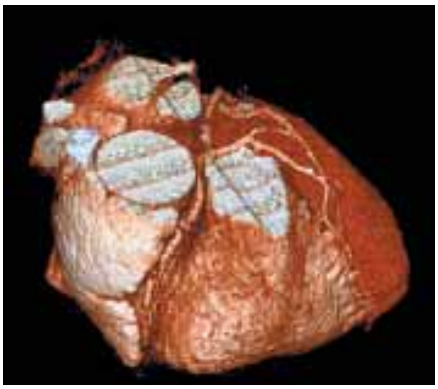
Dr. med.
Achim Häussler

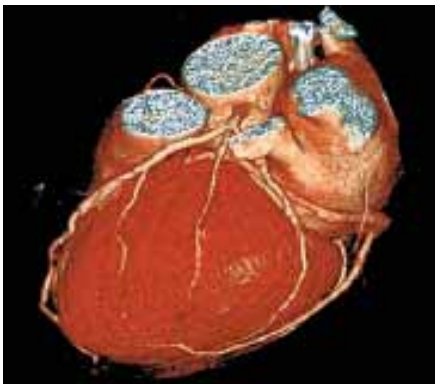
Dr. med.
André Plass

Robotics and Innovative Technologies in Cardiovascular 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
- Device development for beating heart coronary artery bypass operations

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 2004

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

Collaborations:

- Department of Radiology, University Hospital Zürich (Simon Wildermuth, 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)
- Computer Vision Laboratory, ETH Zürich (Prof. Gabor Szekely)

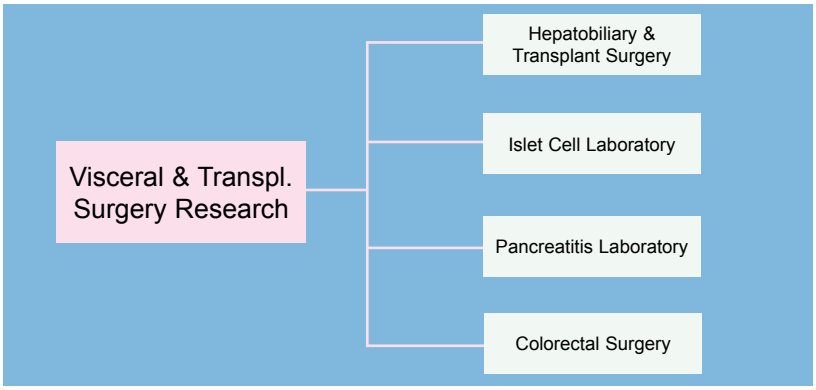
Selected references:

- Cattin P, Dave H, Grunenfelder J, Szekely G, Turina M, Zund G. Trajectory of coronary motion and its significance in robotic motion cancellation. Eur J Cardio-thorac Surg 2004;25(5):786-90
- Boutsianis E, Dave H, Grunenfelder J, Frauenfelder T, Ventikos Y, Wildermuth S, Turina M, Zund G. Computational simulation of intracoronary flow based on real coronary geometry. Eur J Cardio-thorac Surg 2004;26(2):248-56

2.2 Visceral & Transplant Surgery Research

PD Dr. phil II
Rolf Graf

Prof. Dr. med.
Alain Clavien



2.2.1 Hepatobiliary & Transplant Surgery

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

PD Dr. med.
Yinghua Tian

Dr. med.
Nazia Selzner

Dr. med.
Panco Georgiev

Dr. med.
Harm Hoekstra

Dr. des.
Ashraf Osman

Dr. nat.
Jae-Hwi Jang
PhD

Udo Ungethüm

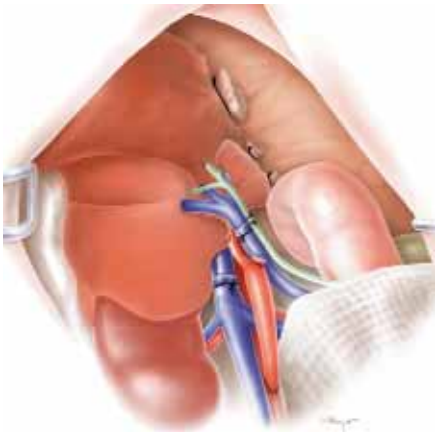
Ischemia / Reperfusion Injury and Liver Transplantation
PD Dr. med. Yinghua Tian; PD Dr. med. Markus Selzner, Dr. med. Nazia Selzner, Dr. med. Harm Hoekstra, Dr. med. Panco Georgiev, Dr. des. Ashraf Osman

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.

Intravital microscopy was used to identify the cellular mediators of ischemia reperfusion injury. The microvascular function, including perfusion rates, leukocyte adherence and Kupffer cell phagocytic activity was assessed in a model of warm ischemia. We could show that early after reperfusion of the ischemic liver, microvascular changes indicated a deterioration of Kupffer cell activity and an increase leukocyte-endothelial interactions. Protective strategies e.g. ischemic preconditioning and intermittent clamping could improve these parameters.

Ischemia reperfusion injury was studied in two types of diseased liver and in the old livers. In the fatty livers we demonstrated that the tolerance of macrovesicular steatosis to ischemic injury was worse than in livers with microvesicular steatosis. The perturbance of microcirculation is one potential mechanism explaining this difference.
Old livers have also a reduced tolerance to warm ischemic injury compared to young livers. Ischemic preconditioning s not an effective protective strategy in the old livers, while in the young we observe a signifcant protection. Low ATP levels in the old livers might be responsible for these effects because reconstitution of ATP levels improved the ischemic insult in the old liver.

Using sophisticated models of liver transplantation in mice and rats, we investigated mechanisms of injury induced by cold ischemia. Our studies indicated that cellular blood elements (i.e. leukocytes, platelets) are critical in inducing apoptosis in sinusoidal endothelial cells. Further development of the mouse model of transplantation included the question whether the small-for-size syndrome could be mimicked in this liver transplantation model. Indeed, 30% liver grafts do not survive representing a situation similar to the clinical experience. Efforts to improve survival of such grafts resulted in the identification of new therapeutic approaches included the application of pentoxifylline, drug targeting microvascular function and Kupffer cell activity.



Schematic representation of the anastomoses after an orthotopic liver transplantation in the mouse

In another project, the effect of toxic bile on cold reperfusion injury was assessed. In a model of phospholipid depletion (Mdr2 knockout mice) we could show that bile composition affects the pathology of bile duct after transplantation.

To evaluate the effect of cholestasis on surgical intervetions, several models of total and partial bile duct obstruction were established. Mice used in these experiments exhibited various levels of bilirubin, indicators of cholestasis. We could show that cholestatic mice were protected from ischemic injury. The mechanism by which this protection is conferred is currently under investigation.

Achievements 2004

- We used a unique model of arterialized partial liver transplantation in mice. We could show that small-for size livers (30%) do not survive while transplantation of 50% of the liver resulted in 100% organ survival. In the 30% small-for-size liver, thereapeutic approaches led to the detetion that pentoxifylline could rescue the small-for-size syndrome. The improvement was based on better microvascular parameters as detec ted by intravital microscopy.
- Establishment of mouse models of cholestasis
- The role of ATP and energy levels during organ preservation and transplantation is still controversial. We investigated the presence of ATP in the steatotic mouse liver. We could show that ischemic preconditioning, a surgical procedure is protective and increases ATP levels.
- Investigations into the mechanism of ischemia reperfusion injury in fatty livers led to the conclusion that these livers were more susceptible to injry. Protective strategies such as ischemic preconditioning could rectify the damage in fatty livers.

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.
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  (2004) Liver Transplantation 10, 520-528.
- 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. Submitted to Hepatology.

Dr. med.
Nazia Malekkiani
Selzner

Dr. med.
Mickael Lesurtel

Dr. med.
Stefan Heinrich

Liver regeneration

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

The liver is the only solid organ with the ability to regenerate to its initial size, which is a critical process after major liver resections. While several intracellular mediators of regeneration have been discovered during the past decade, the extracellular mediators of hepatocyte regeneration are still unknown. In rodent models using partial hepatectomy or partial liver transplantation, we were able to further dissect the complex extracellular mechanisms including the involvement of cytokines and the interaction of various cell types in the sinusoids.

Animal studies in the steatotic liver indicated that the process of liver regeneration is significantly impaired at several levels of the pathway.

Also, we were able to show in a mouse model, that the aging liver has a decreased capacity to regenerate. These findings may open novel avenues for treatment of patients with hepatic steatosis and elderly patients.

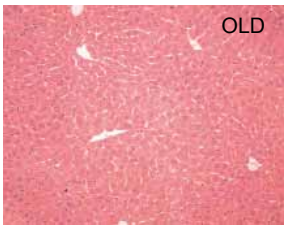

Fig. 1a

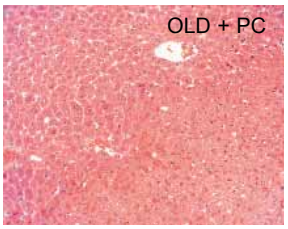

Fig. 1b

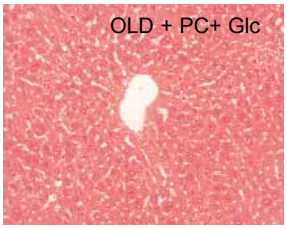

Fig. 1c

Old mice have a decreased tolerance to ischemic injury of the liver with presence of necrosis after 60 minutes of ischemia and 4hr of reperfusion. (Fig. 1a). Ischemic preconditioning is not protective in old mice and increase the necrosis of the liver (Fig. 1b). Pretreatment of old mice with intravenous glucose significantly improve the injury of the liver with almost complete regression of necrosis (Fig. 1c).

Initial processes responsible for a co-ordinated process of cellular proliferation include the activation of cytokines and a highly ordered induction of proliferation of hepatocytes, cholangiocytes and endothelial cells. Prior to regeneration, the liver may be exposed to a short period of ischemia followed by reperfusion of the organ. We have previously demonstrated that platelets are sequestered during reperfusion resulting in endothelial cell injury.

Since a molecular interaction between platelets and liver cells seems to occur during a critical time during initiation of regeneration we asked whether platelets may play a role in the initiation of proliferation and regeneration. We tested this hypothesis in mice, using a model of platelet depletion i.e. thrombocytopenia. In mice injected with an antibody directed against platelet epitope, the platelet count was strongly reduced (Fig.2a). After a partial hepatectomy, the number of proliferating cells (Ki67 stain) were significantly reduced (Fig.2b) suggesting that liver regeneration was strongly impaired in these mice.

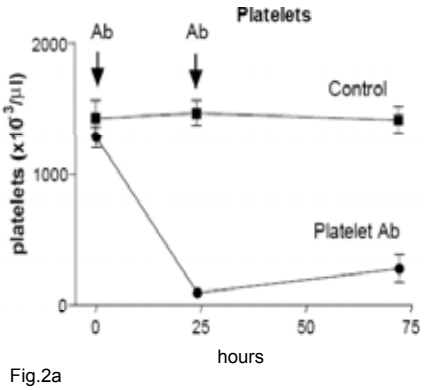

Fig.2a

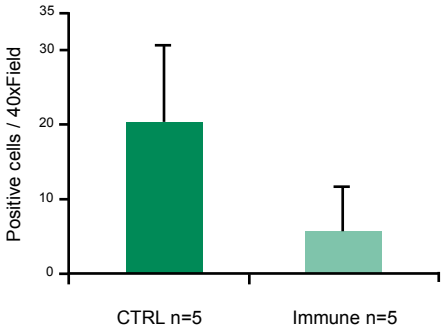

Fig.2b

Achievements 2004

Scientific

- Establishment of a new model thrombocytopenia

Personnel

- Dr. Nazia Selzner received the young investigator award from the Gastroenterological Society (Geneva)

Collaborations:

- PD. Dr. B. Odermatt, Labor für Molekulare Diagnostik, Institut für klinische Pathologie
- Prof. Chr. Gachet, INSERM 311 EFS Alsace 67065 Strasbourg
- Prof. M Bader, Max Delbrück Center for Molecular Medicine, Berlin

Selected references:

- Tian Y, Graf R, Jochum W, Clavien PA. Arterialized partial orthotopic liver transplantation in the mouse: a new model and evaluation of the critical liver mass. Liver Transpl 2003, 9:789-95.
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- Selzner N. Selzner M. Tian Y. Kadry Z. Clavien PA. Cold ischemia decreases liver regeneration after partial liver transplantation in the rat: A TNF-a/IL-6-dependent mechanism. Hepatology 2002, 36: 812-8.

Dr. med.
Stefan Heinrich

Dr. med.
Felix Dahm

Dr. med.
Daniel Dindo

Oncology

Dr. med. Stefan Heinrich, Dr. med. Daniel Dindo, Dr. med Felix Dahm

In our laboratory, we have approached cancer treatment with a new strategy, by using ceramides which constitute components of the lipid bilayer. Ceramides can be chemically modified to specifically target mitochondria and potentially damage these organelles. In cell culture assays we have been able to show that bromated long-chain ceramides specifically insert into the mitochondria. Subsequent cell death causes a reduction in cell number.

This novel strategy is now being tested in animal experiments to explore whether the progression of preexisting metastatic lesions can be interrupted. Using BALB/c mice and an established syngeneic cell line (CT-26), we were able to establish a reliable model for liver metastases by intrasplenic cell inoculation. In addition, we established a model for lung metastases by intravenous injection of the cells into the penile vein.

In a second project, the effect of liver regeneration on liver metastasis was investigated. The clinical background is the question whether liver regeneration after liver resection or portal vein ligation promotes tumor growth in the remaining lobes. To explore this question, a mouse model of partial hepatectomy or portal vein ligation was used. Animals were injected with a syngeneic colorectal cell line followed by liver resection or portal vein ligation 7 days later. Regeneration and metastatic mass was evaluated. We could show that liver regeneration after portal vein ligation was normal, while liver regeneration was significantly lower after liver resection in mice with liver metastases. In addition, metastatic mass was also less after resection than after portal vein ligation.

In another project, we investigated whether water could be used to induce metastatic cell death. For years, surgeons have washed the abdominal cavity with distilled water to lyse isolated cancer cells left after surgery. To evaluate whether this strategy is a useful approach, we determined the mechanism of cell death after exposure to water. Our study demonstrated a novel pathway of cell death by apoptosis in human colon cancer cells following a short hypotonic stress. This pathway is induced by transitory cell swelling which leads to extracellular release of adenosine triphosphate (ATP) and specific binding of ATP to P2 receptors.

Achievements 2004

Scientific

- Establishment of in vitro and in vivo tests for ceramide treatment of tumors

Personnel

- Dr. D. Dindo received an award from the Swiss Society of Surgery

Collaborations:

- Prof Dr. Y. Hannun, Dept. of Biochemistry and Molecular Biology, University of South Carolina, Charleston, USA

Intra- and extramural activities

- As part of our training program for the fellows in the laboratory, international scientists (visiting professors) were invited for discussion of projects and to give a seminar in the weekly Surgical and Gastroenterological GrandRound.
- We organized the third Hepatobiliary and Gastrointestinal Research Retreat in Vulpera, in which several groups from Zürich (Prof. G. Kullack-Ublick, Gastroenterology, Prof. P. Meier-Abt, Clinical Pharmacology and Toxicolgy, and Prof. P.-A. Clavien, Visceral and Transplant Surgery), from Bern (Prof. J.-F. Dufour) and Basel (Prof. M. heim) from Zürich participa ted. In addition, internationally recognized scientist were invited to give le-tures summarizing research from their own laboratory.

Selected references:

- Selzner M. Bielawska A. Morse MA. Rudiger HA. Sindram D. Hannun YA. Clavien PA. (2001). Induction of apoptotic cell death and prevention of tumor growth by ceramide analogues in metastatic human colon cancer. Cancer Research 2001, 61:1233-40.
- Selzner, N. Selzner, M. Graf, R. Ungethuem, U. Fitz, J. G. Clavien, P. A. Water induces autocrine stimulation of tumor cell killing through ATP release and P2 receptor binding. Cell Death Differ 2004, 11 Suppl 2, S172-80

2.2.2 Islet Cell Laboratory

PD Dr. med.
Markus Weber

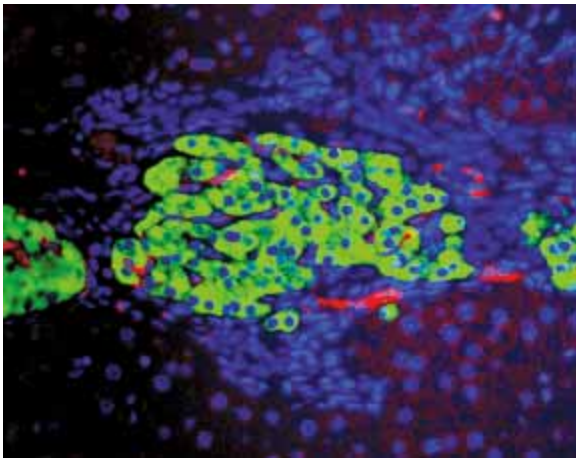
Dr.phil. II
Wolfgang Moritz

Lu Tuyet Trinh

Improvement of cell survival 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 four 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. But equally important, we have shown, that due to their devascularized state, isolated islets undergo massive cell death which is pronounced in the central areas and probably a consequence of diffusion-limited oxygen and nutrient supply. 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 14 days after syngeneic, intrahepatic transplantation. Section was stained for insulin (green), microvascular endothelium (red) and nuclei (blue).

Achievements 2004

Previously, we have been able to demonstrate by in vitro studies that hypoxia is detrimental to islet cell viability and survival. We developed an in vivo transplantation model with non-diabetic, syngenic rats to study the engraftment process within the immediate phase after transplantation which is independent of immunological and metabolic constraints but might be related to long term ischemia. Isolated islets are administrated by portal injection and graft survival was assessed by histological examination at various time points after transplantation. This allowed us to gain important knowledge about post-transplantation events, such as focal necrosis of the liver parenchyma, macrovesicular steatosis and the onset of liver regeneration. Signs of revascularization of islets can be observed as early as day 3 post transplantation which seems to be facilitated by a general remodelling process of islet architecture. In general, loss of transplanted islet mass was less than what could have been expected based on our in vitro studies. Further experiments are now planned to evaluate the graft survival in a diabetic state of recipient rats.
Difficulties to accurately assess graft mass at different time points after transplantation let us to develop new strategies for non-invasive monitoring of functional islet graft. For this purpose we generated adenoviral vectors bearing two different reporter genes that are intended to be introduced into isolated islets prior to transplantation, whose gene products then can be detected either in the serum or by positron emission tomography (PET). Both read outs will hopefully correlate to functional islet mass and allow to follow the engraftment process without the need to sacrifice the recipient animal. Experiments to prove this concept are ongoing.
This project is part of the Swiss National Science Foundation research grant that has been approved in 2002. 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. PET studies are performed in collaboration with PD Dr. S. Ametamey and Dr. M. Honer from the Center of Radiopharma ceutical Research at the Paul-Scherrer Institute in Villigen AG.

Selected references:

- Moritz W., Meier F., Sroka 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: *in press*

2.2.3 Pancreatitis Research Laboratory

PD Dr. phil. II
Rolf Graf

PD Dr. med.
Daniel
Bimmler

Dr. rer. nat.
Li K. Sun

Dr. med.
Franco Fortunato

Dr. med.
Marc
Schiesser

Dipl. phil. II
Theresia
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Philippe
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PD Dr. Rolf Graf, PD Dr. med. Daniel Bimmler

Animal models of chronic pancreatitis

Models for chronic pancreatitis are scarce. One of the better models is the spontaneous development of pancreatic inflammation and fibrosis in the WBN/Kob rat. These animals exhibit signs of parenchymal destruction, inflammation, fibrosis and finally diabetes. Studies by other groups have shown that proinflammatory cytokines such as TNF-α and Il-6 as well as INF-γ and TGF-β are activated during the early phase of the disease. To test whether anti-inflammatory drugs reduce inflammation and subsequent fibrosis, we fed rats a diet containing Rofecoxib. Animals were treated over a period of almost a year. Several parameters, including morphological scores, cytokine mRNA and collagen deposition were vastly improved, suggesting that initial inflammation and subsequent fibrosis were a continously linked process.

Analysis of secretory stress proteins during pancreatitis

Pancreatic stone protein (PSP/reg) and pancreatitis-associated protein (PAP) belong to a family of conserved proteins predominantly expressed in the pancreas. Synthesis and secretion during stress and disease are highly increased and suggest that they belong to the acute phase reaction. Our laboratory developed isoform-specific ELISAs against rat PSP/reg and PAP I, II & III. These diagnostic tools are used to understand the regulation of PSP/reg and PAP in animal models of chronic pancreatitis (WBN/Kob rat). In this rat, all isoforms are significantly up-regulated during the inflammatory process. Acinar cells which produce these proteins change their morphology with a breakdown of the normal architecture. The loss of acinar function eventually leads to diabetes.

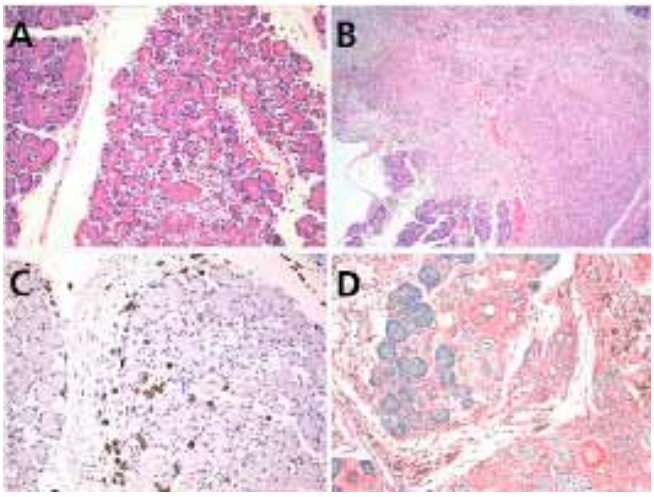


Fig. 1
Histological evolution of chronic pancreatic inflammation. A: early inflammatory infiltration with most of the parenchyma still intact. B: late, chronic inflammation and fibrosis. C: demonstration of macrophage infiltration. D: Sirius red stain for the demonstration of collagen fibrils, representing fibrotic changes.

Achievements 2004

- Scientific

1) We have been able to establish specific antibodies and design ELISAs for the detection and determination of human and mouse pancreatic secretory stress protein isoforms. These diagnostic tools are currently used in a pilot project in conjunction with clinical assessment of stress and disease relating to pancreatic inflammation.
2) The demonstration of the pathology of the secretory apparatus in the WBN/Kob rat using a model of chronic pancreatitis.
3) Successfully reduce inflammation and fibrosis with a COX-2 specific inhibitor.

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:

- Bimmler, D. Schiesser, M. Perren, A. Scheele, G. Angst, E. Meili, S. Ammann, R. Graf, R. (2004) Coordinate regulation of PSP/reg and PAP isoforms as a family of secretory stress proteins in an animal model of chronic pancreatitis. J Surg Res 118, 122-35
- Reding, Th Sun, L. K. Perren, A. Bain, M. Bimmler, D. Graf, R. (2004). COX-2 dependent amelioration of inflammation in an animal model of chronic pancreatitis. Pancreas, 29, 352

2.2.4 Colorectal Surgery

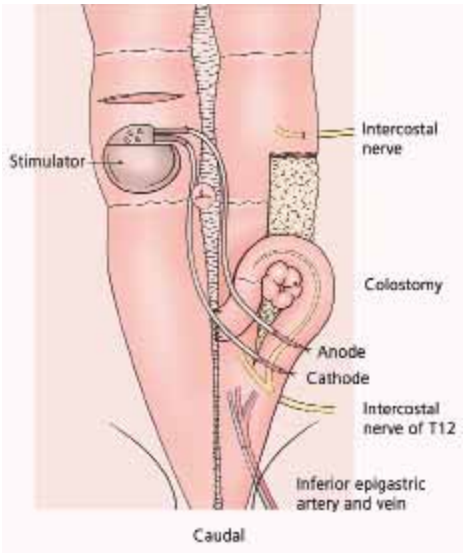
Dr. med.
Franc Hetzer

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

med. prakt.
Elenora Brunner

Dr. Franc Hetzer MD, Elenora. Brunner, Dr. Dieter Hahnloser MD,
PD Dr. Nicolas Demartines

Every year, around 600 patients receive new permanent colostomies in Switzerland. The figures for the United States are 100 000. At least 50% of these patients have a life expectancy of more than five years at the time of the procedure. Stoma patients experience a clear decrease in quality of life with several limitations of their private and professional activities, e.g. continuous involuntary bowel movement or fear of bad smells or leakage from the stoma bag. Various techniques such as irrigation or colostomy plug are available but are only regularly used by one-third of patients.
At present, there is no surgery that would significantly improve the quality of life of stoma patients. Following the success of the dynamic graciloplasty the creation of a neosphincter around a stoma using similar principles merits investigations.
In a pilot study we demonstrated the feasibility of a continent stoma in an animal model with a dynamic rectus neosphincter in 2002. A distal based rectus muscle sling surrounding the stoma 270 degrees and a low frequency conditioning schema achieved a continent colostomy for more than 12 hours during 5 days. The neosphincter profile revealed a 40 mm segment of high pressure, with mean of 74 mmHg (range 67-82 mmHg). Type I muscle fibres increased from 38% (32-42%) to 74% (66-78%) after 12 weeks conditioning.
In six mini pigs the results of continent stoma was confirmed by Ms E. Brunner and Dr. F. Hetzer in 2004. The results are going to be published soon.



Schematic illustration of dynamic graciloplasty

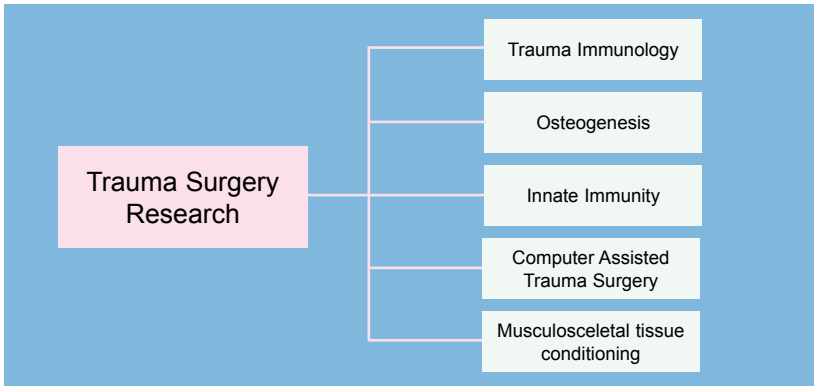
Selected references:

- Hetzer FH, Künzi W, Schwizer W, Demartines N. Continent colostomy with rectus abdominis neosphinc-teroplasty: development of an animal model. Br J Surg, 2003;90:1273-9

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.
Ladislav Mica

Ursula
Steckholzer

Immunomodulation in polytrauma patients
PD Dr. med. Marius Keel MD, Dr. rer. nat. Luc Härter

Intracellular mechanisms regulating neutrophil apoptosis during inflammation and sepsis

The reduced apoptosis of neutrophil granulocytes (PMN) contributes to the pathogenesis of systemic inflammatory response syndrome (SIRS), sepsis and multiple organ dysfunction syndrome (MODS). Endotoxins, like LPS activate NF-κB and reduce apoptosis in neutrophil granulocytes. A hallmark of apoptosis is the activation of caspase-3. The intracellular inhibitor of apoptosis proteins (IAP) have been shown to inhibit activated caspase-3. Therefore, the role of NF-κB in LPS-mediated PMN survival was investigated in patients with sepsis. Furthermore, the turnover dynamics of cIAP-2 and caspase-3 protein were investigated in PMN.
PMN (1 x 10^6/mL) were preincubated for 1 hour with a specific NF-κB-inhibitor (SN50, 20 μM), a proteasom inhibitor (PSI, 30 μM), or medium alone and then stimulated with or without LPS (1 μg/mL) for 15 hours. Apoptosis was quantified by Annexin-V and propidium iodide staining by flow cytometry (FACS) and IL-8 release measured in ELISA. For cIAP-2 induction PMN from healthy volunteers were preincubated with endotoxin (LPS, 1 μg/mL) for 5 hours, followed by an additional hour with or without PSI (30 μM), before incubation with or without agonistic CD95 antibody (100 ng/mL) for another 16 hours. Caspase-3-activity was determined by DEVD-afc-cleavage assay. Expression of ubiquitinated caspase-3 and cIAP-2 protein was detected by Western-blot analysis and cIAP-2 mRNA by RT-PCR.
LPS significantly ($P<0.05$) reduced spontaneous (66.1 ± 2.3% to 24.8 ± 4.8%) and CD95-induced (90.8 ± 0.9% to 64.3 ± 4.2%) apoptosis and caspase-3 activation. Inhibition of NF-κB had no effect on spontaneous apoptosis in control or septic PMN, nor on LPS-induced survival, whereas inhibition of the proteasom completely abolished the antiapoptotic effect of LPS on spontaneous (52.6 ± 2.4%) and CD95-induced (88.7 ± 2.6%) apoptosis, as well as degradation of caspase-3.

However, SN50 significantly ($p < 0.05$) inhibited LPS-induced IL-8 secretion in controls and patients with sepsis.
In addition LPS induced cIAP-2 mRNA, as well as protein within two hours incubation. Furthermore, LPS increased ubiquitination of activated caspase-3 as was seen in westernblot.
From these findings we conclude, that although NF-κB is activated after LPS stimulation, NF-κB is not involved in the regulation of apoptosis. Induction of cIAP-2 by LPS and accelerated degradation of activated caspase-3 by the proteasom might be responsible for the reduced apoptosis in PMN during sepsis.

Achievements 2004

- Schweizerischer Nationalfonds (SNF) grant approval (3200BO-105987) for M. Keel, L. Labler, L. Härter: Wound healing in VAC®(vacuum assisted closure)-treated, injured patients: Implications of neutrophil activation for accelerated angiogenesis.

Kongressbeiträge:

- L. Härter, L. Mica, O. Trentz, M. Keel. Upregulation of toll-like receptors in neutrophils is regulated by STAT-3. Day of Clinical Research USZ 26-27.03.2004 Zürich
- L. Mica, L. Härter, O. Trentz, M. Keel. STAT-3 regulates the reduced apoptosis in neutrophils from patients with sepsis. Day of Clinical Research USZ 26-27.03.2004 Zürich
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- Mica L, Härter L, Trentz O, Keel M. Regulation of neutrophil apoptosis in patients with sepsis by STAT 3.
  2. Jahreskongress der Schweizerischen Gesellschaft für Chirurgie 23-25.06.2004 Davos
- Labler L, Lustenberger T, Lüthi S, Mica L, Trentz O, Keel M. Hemorrhagic shock increases infections of closed and open fractures.
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  6th European Congress of Trauma and Emergency Surgery, 9-12.09.2004 Rotterdam

- Mica L, Lustenberger T, Lüthi S, Labler L, Trentz O, Keel M. The Severity of Injury and Hemorrhagic Shock Correlates with the Incidence of Posttraumatic Infectious Complications.
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  3rd Swiss Apoptosis Meeting 16-17.09.2004 Bern
- Härter L, Mica L, Trentz O, Keel M. STAT-3 participates in endotoxin-induced survival in neutrophils from patients with sepsis.
  3rd Swiss Apoptosis Meeting 16-17.09.2004 Bern
- Keel M. Polytrauma Zurich: Trauma System, Management Concept and Research. Trauma and Surgical Intensive Care Unit, LAC+USC Medical Center, Los Angeles, November 2004.

Collaborations:

- Dr. med. Florian J. Jung, PD Dr. med. Stephan Korom, Division of Thoracic Surgery, USZ, Zürich
- PD Dr. R. Graf, Division of Visceral & Transplant Surgery, USZ, Zürich

Selected references:

- Härter L, Mica L, Stocker R, Trentz O, Keel M. Increased expression of toll-like receptor-2 and -4 on leukocytes from patients with sepsis.
  2004 Shock 22(5):403-409.
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  2004 J Pineal Res. 37(1):36-41.
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2.3.2 Osteogenesis Laboratory

Dr. med. O. A. Trentz, Dr. med. M. Egermann, Dr. med. A. E. Handschin, S. Hemmi

Dr. med. Omana A. Trentz

Dr. med. Marcus Egermann

Dr. med. Alexander E. Handschin

Sonja Hemmi

Heterotopic Ossification

Heterotopic ossifiaction (HO) is defined as ectopic bone formation at sites that do not normally ossify. Mature HO shows cancellous bone with harversian canals, cortex, blood vessels and bone marrow with only a minor amount of hematopoesis. Paraarticular and intramuscular bone formations cause restricted range of motion, pain and ankylosis. Mature ossifications can be resected, but with a high recurrency rate. The onset of HO usually occurs after several months, but its induction probably starts immediately following the initial trauma. We have observed early changes of bone metabolism following trauma. Because osteocalcin is an important regulator of bone metabolism, we focused our study on osteocalcin and other phenotype marker expression in osteoblasts from both iliac crest and HO. The lower levels of osteocalcin detected in our study may play an important role in patients with central nervous system and the later development of posttraumatic heterotopic ossification.

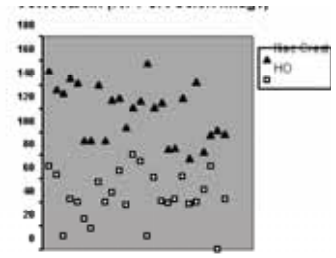


Figure 1: Osteocalcin expression in osteoblasts from heterotopic ossifications (white box) and from iliac crest (black triangle). Osteocalcin expression is significantly depleted in cells originating from ectopic bone (HO).

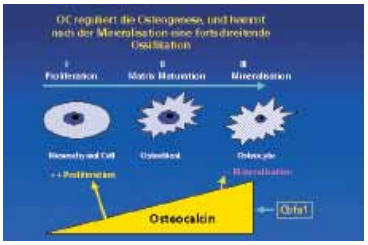


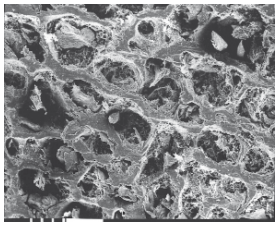
Figure 2: Osteocalcin regulates osteoblast proliferation and mineralization.

Figure 3: Cbfa-1 is the transciption factor for osteocalcin. Cbfa-1 knock out mice show a complete lack of ossification.

Tissue Engineering

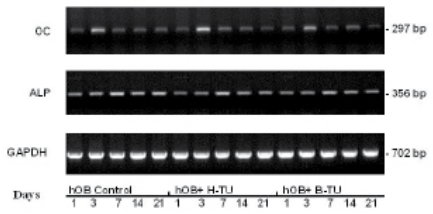
The repair of large osseous defects remains still an unsolved problem in bone surgery. The use of autogenous bone grafts is widely accepted and considered to be the golden standard in the treatment of bony defects. However the disadvantage of autogenous bone grafts are limited availability, harvesting morbidity and insufficient biomechanical properties. The problems with autografts have initiated the development of several allogenic, xenogenic and synthetic bone graft alternatives. Their complication rate due to interaction between biomaterials and host tissues could be reduced, but still cell-mediated immune responses, and synthesis and resorption by osteo-blasts resp. osteoclasts are not yet fully solved.
We investigate the biocompatibility of bone substitutes using standardized in-vitro testing. In addition to heal large osseous defects we evaluate the effect of various growth factors on cell cultures of osteoblast.

Tutoplast Disc



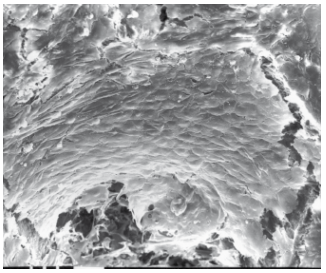
Tutoplast SEM (1000 µ) without cells

Angiogenesis in chicken embryo
(in Chorio-allantoin-membrane (CAM)



RT-PCR: Osteocalcin (OC) and ALP

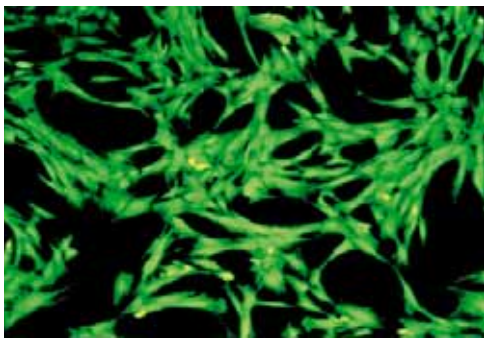
Day 7 on Tutoplast



Day 21 on Tutoplast

Effect of antithrombotic drugs on primary human osteoblasts in vitro

Prophylaxis and treatment of venous thromboembolism may require long-term heparin therapy. The prolonged administration of heparin has been associated with an increased risk of heparin-induced osteoporosis. This effect was also demonstrated in *in vitro* studies on osteoblasts. Fondaparinux (Arixtra®) is a new antithrombotic drug, which, in contrast to heparin preparations, is a full synthetic, single chemical entity that has the ability to specifically inhibit factor Xa. Fondaparinux-sodium has been recently approved by the Food and Drug Administration (FDA) in the prevention of venous thromboembolism in orthopaedic surgery after proven efficient in four phase III trials and is increasingly used in many orthopaedic surgery centres. However, critics of the available trials point out, that more data and experience, including potential side-effects, are required before the role of fondaparinux in thromboprophylaxis after major orthopaedic surgery is established with confidence. Because of the known interactions of other antithrombotic and anticoagulatory agents on bone remodelling, we wanted to analyse the effect of fondaparinux on human osteoblasts in vitro and compare them to the effect of heparin.

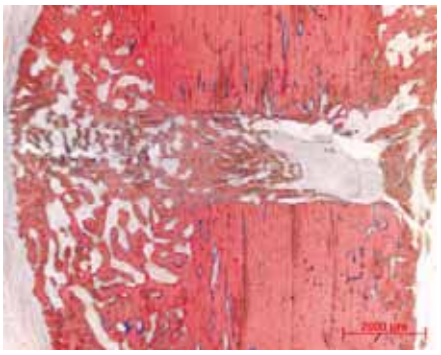


Human osteoblasts under heparin incubation

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 biochemanical properties of new formed bone leading to high risk of fixation failure. 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. The objective of this study is to investigate the fracture healing in a small animal model for osteoporosis (SAMP-6 mice) and study the influence of BMSC from osteoporotic and healthy donors on bone healing.

New formed bone within the fracture gap at 8 weeks during fracture healing.



Callus formation at 8 weeks during fracture healing using an ovine fracture model.

Achievements 2004

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Collaborations:

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- PD Dr. med. HJ Kock, Unfallchirurgie, Hochtaunuskliniken Bad Homburg
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- Prof. Erich Schneider, Prof. Mauro Alini, AO Research Institute, Davos, Switzerland
- Prof. W. Hofstetter, Departement of Clinical Research, University of Berne

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- Dahlman TC. Osteoporotic fractures and the recurrence of thromboembolism during pregnancy and the puerperium in 184 women undergoing thromboprophylaxis with heparin. Am J Obstet Gynaecol, 1993, 168:1265-70
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2.3.3 Innate Immunity Laboratory

PD Dr. med.
Michael
Heinzelmann

Dr. med.
Herbert Bosshart

Modulation of Host Responses to Bacterial Endotoxin
PD Dr. med. Michael Heinzelmann; Dr. med. Herbert Bosshart

Lipopolysaccharide (LPS) is unique to Gram-negative bacteria. Mammals are equipped with an LPS-sensing machinery consisting, primarily, of LPS-binding protein (LBP), CD14 and Toll-like receptor 4 (TLR4). Modest stimulation of TLR4 facilitates the elimination of invading microorganisms. Potent TLR4 stimulation, however, produces severe reactions in the host, often leading to multi-organ failure and death. Our research focuses on the identification of synthetic or naturally-occurring LPS-modifying substances. Specifically, we aim to elucidate the molecular mechanisms by which these substances interfere with LPS activation. These efforts are ultimately geared towards the development of novel anti-sepsis drugs.

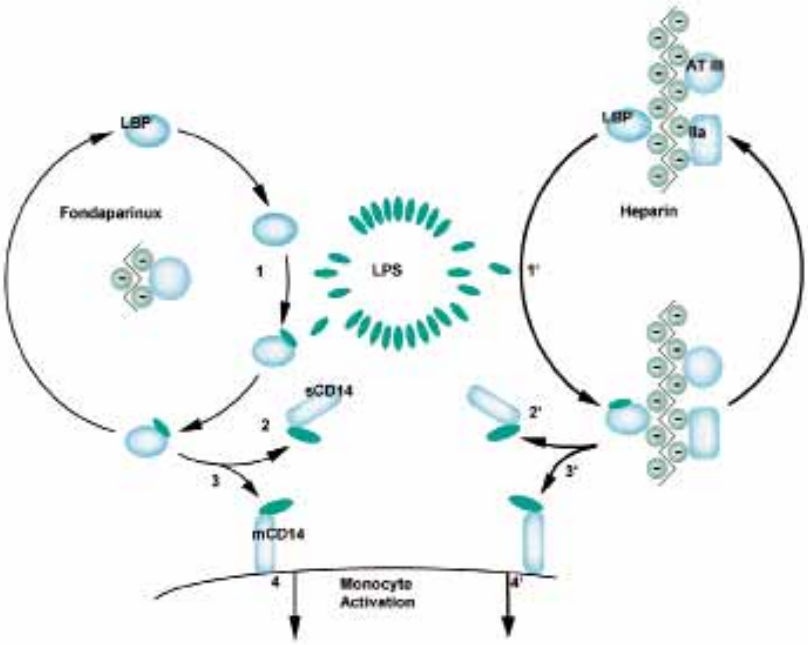

Figure
A model for the interaction between heparin and LBP. LPS micelles inefficiently activate $CD14^+$ monocytes. LBP disaggregates LPS (1) and catalyses the transfer of LPS monomers to either soluble (2) or membrane-bound CD14 (3). Subsequent activation of monocytes (4) is thought to occur through a physical interaction between membrane-bound CD14-LPS and MD2-TLR4 complexes. We propose that binding of LBP to heparin enhances the catalytic ability of LBP to transfer LPS (1', 2', 3'), thereby resulting in the amplification of LPS signalling (4'). Other heparin-binding plasma proteins such as antithrombin III (AT III) or thrombin (Factor IIa) will also bind to heparin. Plasma concentrations of heparin, LPS, LBP and soluble CD14 (sCD14), as well as expression levels of membrane-bound CD14 (mCD14) may determine to what extent LPS signals are amplified. Fondaparinux, which is identical to the AT III-binding pentasaccharide sequence in heparin, does not interfere with LPS-induced monocyte activation.

Achievements 2004

- We have identified LPS-binding protein (LBP) as a novel heparin-binding plasma protein. The affinity of LPB to heparin was KD = 55 ± 8 nM, as measured by surface plasmon resonance. Using a fluorescence-based assay, we showed that clinically used heparin preparations significantly enhance the ability of LBP to catalytically disaggregate and transfer LPS to CD14, the LPS receptor. The presence of clinically relevant heparin concentrations in human whole blood increased LPS-induced production of the proinflammatory cytokine IL-8. Fondaparinux, which is identical with the antithrombin III-binding pentasaccharide in heparin, did not bind to LBP or alter LBP function. Thus, this novel anticoagulant drug is a potential candidate for safe administration to patients who have endotoxemia and require anticoagulation

  LPS-enhancing effects are not unique to heparin or heparin-like structures. We found that arginine-rich cationic polypeptides, such as human CAP37 or the small structurally related salmon protamines, also enhance LPS-induced monocyte activation. The mechanism by which polycations (protamines) act synergistically with LPS differs in a fundamental way from the mechanism employed by polyanions (heparin). Polycations bind to both, LPS and cellular surfaces. Polycations are therefore LBPs which, like liver- derived endogenous LBP, mediate LPS activation of peripheral blood monocytes. These observations support the prediction that other strongly basic proteins could also act as amplifiers of LPS responses.

  Synthetic poly-L-histidines (Hn) have the capacity to neutralize LPS-induced cell activation. We observed that the Hn-mediated LPS-neutralizing effect is tightly coupled to a protonated state. This finding was unexpected because other polycations such as synthetic poly-L-arginines (Rn) or the above-mentioned salmon-derived protamines enhance LPS responses. Our results support a model in which Hn-bound LPS is neutralized, presumably because Hn fails to efficiently transfer LPS to cell surface-localized LPS receptors. We also discovered that a short synthetic histidine-rich peptide, derived from the human histidine- and proline-rich glycoprotein (HPRG), posseses potent LPS-neutralizing properties. Studies to define the role of HPRG in the context of LPS signalling are ongoing.

Collaborations:

- Hans Flodgaard, Leukotech, Fruebjergvej 3, Box 8, 2100 Copenhagen - Denmark
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- Hans-Peter Beck, Swiss Tropical Institute, Socinstrasse 57, CH 4002 Basel - Switzerland
- Jerome Pugin, Geneva University Hospital Department of Internal Medicine 24, rue Micheli-du-Crest, CH 1211 Geneva 14 - Switzerland

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- Bosshart H, Heinzelmann M. Arginine-rich cationic polypeptides amplify lipopolysaccharide-induced monocyte activation. Infect Immun, 2002; 70: 6904-6910.
- Bosshart H, Heinzelmann M. Endotoxin-neutralizing effects of histidine-rich peptides. FEBS Lett, 2003; 553: 135-140.
- Heinzelmann M, Bosshart H. Fondaparinux sodium lacks immunomodulatory effects of heparin. Am J Surg, 2004; 187: 111-113.
- Heinzelmann M, Bosshart H. Heparin binds to lipopolysaccharide (LPS)-binding protein, facilitates the transfer of LPS to CD14, and enhances LPS-induced activation of peripheral blood monocytes. 2005, J Immunol, 174:2280-7.

2.3.4 Computer Assisted Trauma Surgery (CATS)

PD Dr. med.
Peter Messmer

Dr. med.
Felix Matthews

Dr. med.
Valentin Neuhaus

Dr. med.
Adrian Schwaller

Dr. med.
André Witschi

PD Dr. med. Peter Messmer; PD Dr. med. Guido A. Wanner;
PD Dr. med. Marius Keel, Dr. F. Matthews, Dr. V. Neuhaus, Dr. M. Egermann, Dr. A. Witschi, Dr. A. Schwaller

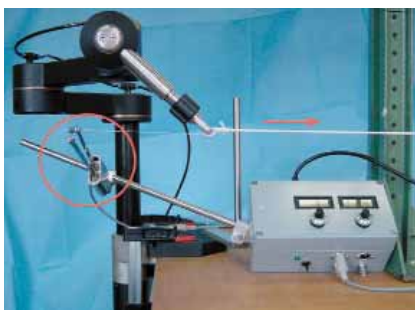
Computer assisted surgery has started with navigation about 15years ago in neurosurgery and soon later in ENT. PD Dr. Wanner and PD Dr. Keel introduced 2D navigation early in 2002 into our clinical work after a phase of training and precision studies. With the liquidation of Medivision there was a period of more than one year of no technical support for laboratory and clinical projects in computer assisted surgery. 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 in Davos and some new industrial partners, such as Siemens Switzerland and Siemens World (Erlangen, D) and BrainLAB (Heimstetten, D). Together with these partners we now cover not only the field of navigation but also technology integration of different aspects and intra-operative imaging in trauma.

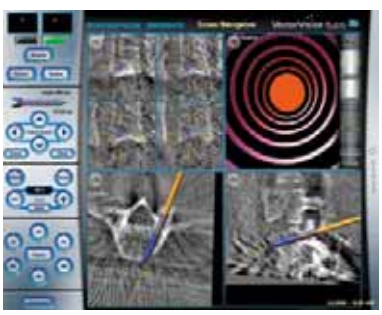
In the biomechanical lab of the orthopaedic university hospital Balgrist we tested stability of a passive holding device for computer aided reduction (Fig 1). Feasability for intra-operative use of the device was tested in the wet lab. The device was constructed by the AO development institute.

For about one year we are exploring the value of 3D fluoro-CT (IsoC3D) for intra-operative judgement of joint reconstructions and implant position. Actually we are designing a project to compare image quality of IsoC3D with computertomography (CT) in joint surgery.

Since we received the new CT-and fluoro-navigation hard- and software from BrainLab only 4 weeks ago we are teaching our group in using the new navigation system together with trainers from the company. It is important to have a group, big enough to support introduction of new techniques in the OR. The new software enables together with IsoC3D to navigate in 3D similar to CT-navigation but only with fluoroscopy. The use of the system in lumbar and cervical spine surgery was rather promising (Fig. 2)

A special module (MEPUC) for navigated positioning of the fluoroscope in the OR was developed by BrainLab to be tested by our group. The project has been approved by the ethical committee and is now tested first in the laboratory environment.





Together with Siemens and CARCAS we are working on a software solution (i3db) to improve workflow from admittance of the patient to preoperative planning, intra-operative handling, optimization of storage, documentation, and accounting. The project involves different divisions of the hospital. A KTI-grant application was submitted.

Six complex cases of sacral non unions or acetabular fractures have been operated in the multifunctional image guided therapy suite (MIGTS) at the university hospital of Basel by the Zurich team using intra-operative CT with modality based navigation.

Achievements 2004

- Establishment of cooperation with industrial partners: Siemens, BrainLab.
- Cooperation with CARCAS Basel continued
- 2D and 3D navigation system of BrainLAB installed.
- First spinal cases (lumbar and cervical spine) operated.
- Approval by the ethical committee for the MEPUC project.
  I3db-KTI-grant application submitted.
- Stability tests of passive holding device performed.

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 der Arbeitsgemeinschaft für Osteosynthesefragen AO/ASIF mit folgenden Partnern:
  - Prof. C. Krettek, Unfallchirurgische Klinik, Medizinische Hochschule Hannover MHH
  - Prof. F. Gebhard. Abteilung für Unfallchirurgie, Hand- und Wiederherstellungschirurgie, 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

Selected references:

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- U. Stöckle, C. Krettek, T. Pohlemann, P. Messmer. Clinical Applications-pelvis. Injury, Int. J. Care Injured (2004) 35, S-A46-S-A56.
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2.3.5 Musculosceletal tissue conditioning

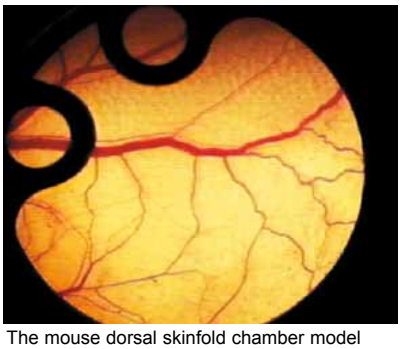
PD Dr. med.
Guido Wanner

Dr. med.
Claudio Contaldo

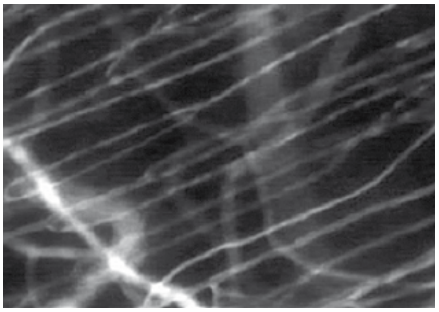
Effects of recombinant human erythropoietin on the microcirculation of critically reperfused striated muscle

Dr. med. Claudio Contaldo; PD Dr. med. Guido A. Wanner

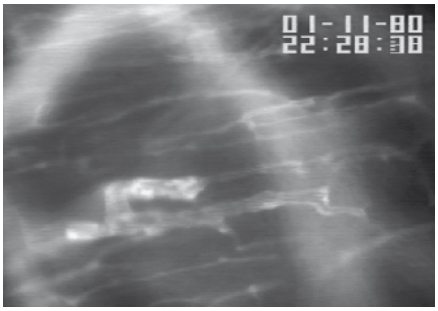
Perioperative microvascular ischemia-reperfusion (I/R) injury of peripheral tissue is characterized by the failure of capillary perfusion and reoxygenation-associated activation of leukocyte endothelium interaction with loss of endothelial integrity, potentially leading to postoperative complications. Erythropoietin (EPO), known for its effects on erythrocyte progenitors, was proposed as a novel cytoprotectant in the brain and the heart following I/R. The objective of our study was to elucidate the impact of EPO in microvascular I/R injury of peripheral tissue. For our investigations we used the mouse dorsal skinfold chamber model, which allows to assess quantitatively the microcirculation *in vivo* by fluorescence microscopy. The microcirculation of striated skin muscle was assessed before 3 h of compartement-induced ischemia (baseline) and 0.5, 2, 6 hours, and 1, 3, and 7 days after onset of reperfusion. Animals were pretreated either 30 min or 24 hours before ischemia with an intraperitoneal injection of Recormon [(5000 IE/ kg KG), recombinant human Erythropoietin]. Reperfusion injury after 6 hours was characterized by the reduction of functional capillary density to 41.3 ± 12 % of baseline ($P < 0.05$, vs. baseline), an increase of macromolecular leakage from 35.2 ± 8 % (baseline) to 79.8 ± 14 % ($P < 0.05$), and a significantly enhanced leukocyte-endothelium interaction in postcapillary venules ($P < 0.05$). Intraperitoneal injection of Recormon 30 min before induction of ischemia significantly attenuated the postischemic reduction of capillary perfusion (68.2 ± 14 %, $P < 0.05$, vs. control), the macromolecular leakage (54.2 ± 6 %, $P < 0.05$, vs. control) and the leukocyte-endothelium interaction in postcapillary venules ($P < 0.05$) after 6 hours of reperfusion. Comparable results in terms of no-reflow and reflow paradox were obtained when EPO was administered 24 hours before onset of ischemia. Regularly, 1-3 days after reperfusion, we observed angioproliferative phenomenons like capillary budding and sprouting, and in many observed fields the formation of new capillary networks after 5-7 days, when EPO was administered 30 min before ischemia. Both timepoints of EPO-pretreatment appeared as capable to significantly attenuate the postischemic no-reflow and to reduce the inflammatory response. Angioneogenesis was only observed when EPO was given 30 min before ischemia. Our study strongly suggests that in the presence of hypoxia EPO treatment can stimulate the proliferation of endothelial cells.



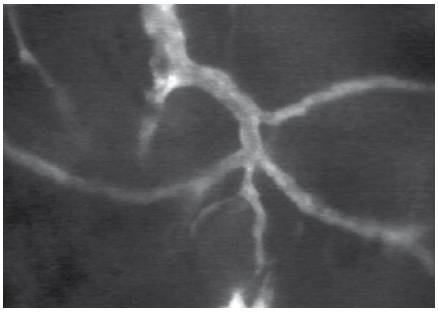
The mouse dorsal skinfold chamber model



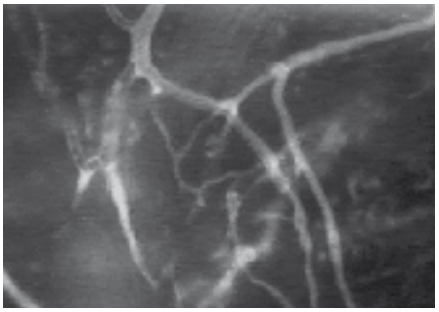
Normal capillaroscopy of striated muscle



Capillary “budding” after EPO treatment (day 1-2)



Capillary sprouting after EPO treatment (day 2-4)



Formation of new capillary networks after EPO treatment (day 5-7)

Achievements 2004

- **Cap-Image**, digital analysis system for the computer-aided evaluation of intravital capillaroscopy

Talks 2004

- The European Association of Plastic surgeons (EURAPS 2004), May 27-29, Genova Italy
  Dr. C.Contaldo: Hemodilution with artificial oxygen carriers attenuates anaerobic metabolism in ischemic hamster flap tissue.

Collaborations:

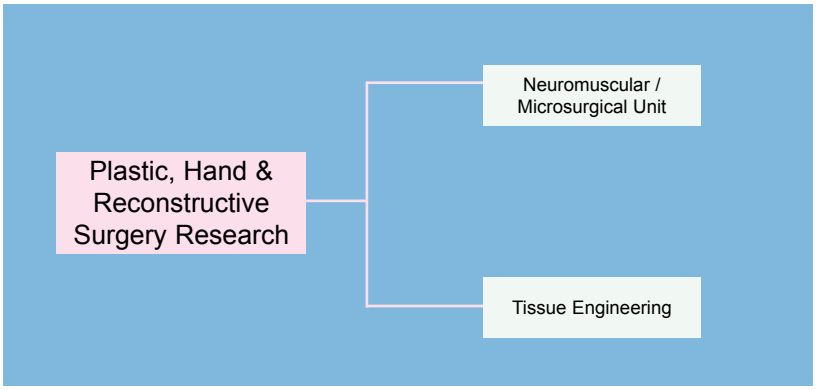
- Prof. Dr. MD Menger, Institut für Klinisch-Experimentelle Chirurgie, Universitätsklinikum, Homburg/Saar, Deutschland

Selected references:

- Contaldo C. et al; The influence of trauma and ischemia on carbohydrate metabolites monitored in hamster flap tissue. *Anesth Analg* (accepted for publication Aug 2004)
- Contaldo C. et al; A new generation of engineered hemoglobins to oxygenate ischemic, collateralized tissue. *Crit Care Med* (accepted for publication Dec 2004)

2.4 Plastic, Hand & Reconstructive Surgery Research

Prof. Dr. med.
Viktor E. Meyer



Dr. med.
Doris Burg

PD Dr. med.
Gertrude Beer

2.4.1 Neuromuscular / Microsurgical Unit

Functional, electrophysiological and morphometric evaluation of nerve regeneration from coaptation on regenerated nerve fibres – experimental studies on the peroneal nerve in the rabbit
PD Dr. med.G.M. Beer, Dr. med. D. Burg

Despite continued research efforts, the results of nerve repair surgery are often discouraging. Among various efforts to improve clinical outcome, one thrust of research has been to identify the optimal site for coaptation in nerve repair. If the surgeon relies on visual observation alone, he or she can only judge the condition of the nerve sheaths but not the quantity or quality of nerve fibres. However, with the advent of intraoperative neurography and the nerve action potential recording, it is possible to judge the quality of the nerve parenchyma during operations. In order to successfully reinnervate a distal nerve stump or a nerve graft, it is commonly assumed that a sufficient number of "good" quality nerve fibres are required. Whereas the importance of the quantity of nerve fibres at a coaptation site is generally acknowledged, the prerequisite "quality" of these nerve fibres has yet to be defined. The question arose whether it is necessary to cut a damaged nerve back to healthy tissue or whether the resection can be restricted to the segment actually impeding regeneration and the coaptation carried out on freshly regenerated nerve fibres.
First studies showed that regeneration is well possible from coaptation on freshly regenerated nerve fibres. Yet, coaptation on regenerated nerve fibres requires a timely separated double lesion, which leads to a nerve conditioning. Currently the influence of conditioning on nerve regeneration is examined.

Use of polyurethane vessels for microvascular training to reduce animal experiments

S.A. Meier, A. Lang, G. M. Beer

To learn microsurgery a systematic training of the manual skills is inevitable. Usually, after first exercises on two-dimensional models the training has continued on animals. With the growing ethic awareness, the obligation to protect animals and the stricter animal protection laws realistic three-dimensional models have become necessary to train microsurgery. Yet, the available alternatives all have certain disadvantages. We tested vessels made of polyurethane for microvascular training and compared them to the available three-dimensional synthetic alternatives.
There are rose (arteries) and blue (venes) coloured, opake vessels with a minimal thickness of the wall of 0.12mm and a minimal internal diameter of 1mm. For mimicking the surgical access and the depth of the operative field the vessels can be embedded in a synthetic box with or without a cap. The completed anastomosis is checked by injection of coloured fluid.
The consistency, and the variable relation of the thickness of the wall to the internal diameter relates very closely the biological situation. Even the training on very fragile venous walls is possible in all manners. After completion of the anastomosis the vessels can be opened longitudinally for checking the patency of the anastomotic site.

Immunohistochemical differentiation and analysis of localization of sweat glands in the axilla of normhidrotic adults.

G.M. Beer, S. Baumüller, N. Zech, P. Wyss, D. Strasser, Z. Varga,
B. Seifert, J. Hafner, D. Mihic-Probst

The histological differentiation of the eccrine and apocrine glands in the axilla with routine stainings has been demanding and this might be one reason why the exact localisation of the glands has been discussed rather contradictorily. In case of hyperfunction of the eccrine glands (hyperhidrosis) or the apocrine glands (bromhidrosis) surgical excision may prove inevitable. The aim of this study is to exactly localize of the glands by use of immuno-histological markers, such as the GCDFP or S 100 protein, for eccrine and apocrine differentiation.
After ethical approval, in 40 normhidrotic adults who underwent axillary surgery a piece of 1 x 1 cm measuring skin was removed for histological evaluation morphometrically. Normhidrosis was evaluated by the "gravimetrical test" (< 50 mg/min).
The study showed that the overwhelming majority of sweat glands are localized in the subcutaneous tissue, near the border to the dermis. As the number and localization of sweat glands are comparable in normhidrotic and hyperhidrotic adults, the results of this study can be applied to the surgical therapy of patients with hyperhidrosis and / or bromhidrosis.

The thoracoacromial vessels as recipient vessels in microsurgery and supermicrosurgery: anatomic and sonographic studies.
G.M. Beer, P. Kompatscher, M. Manestar, A. Lang, V.E. Meyer

The currently most chosen recipient vessels for free flaps in breast reconstruction are the internal mammary vessels and the thoracodorsal vessels. Yet, each of these vessels has certain drawbacks.
The purpose of this study is to determine whether the pectoral branches of the thoracoacromial vessels are suitable as recipient vessels in microsurgery.
The presence and the precise course of the vessels is investigated by anatomical dissection and by colour Doppler ultrasound. In 18 cadavers the pectoral branch of the thoracoacromial vessels is followed caudally until the diameter of the artery diminishes to 1 mm. The same examination is carried out in 40 young female volunteers by tracing the vessel course with colour Doppler ultrasound. The 1 mm and the 2 mm cutoff point of the artery is measured with reference to the manubrium, the midsternal line, the clavicle and the upper border of the most closest rib. At both cutoff points, the diameter of the accompanying vein is measured and the relation to the pectoral nerve recorded.
The study showed that the thoracoacromial vessels are excellently suited as recipient vessels for microsurgery and are a very promising addendum to the thoracodorsal and internal mammary vessels. The results of this study will be applied to the clinical situation.

The bipedicled and the bipartite latissimus dorsi free and perforator flap: An anatomic study.
G.M. Beer, A. Lang, M. Manestar

Although the latissimus dorsi muscle has a dual blood supply with true anastomoses between the dominant thoracodorsal vessels and the secondary intercostal vessels, over the lower one-third of the muscle the blood supply derives largely from intercostal branches which do not connect well with the thoracodorsal branches. In cases where the whole entirety of the latissimus muscle is raised freely on its dominant vascular pedicle, the musculocutaneous unit over the lower one-third may therefore not be consistently viable. Such cases of distal muscle and skin necrosis prompted us to look for an enhanced blood supply in the distal one- third of the latissimus dorsi und thus to have a closer look at the muscular branches of the intercostal vessels. We investigated if one of the lowermost intercostal vessels sends a big enough (> 0.5 mm) muscular branch to the latissimus dorsi to serve as a second vascular pedicle for a bipedicled latissimus dorsi free flap. If so, we further ask, whether it was possible to furnish bipartite muscle, musculocutaneous, or even perforator flaps out of one latissimus dorsi muscle.
The muscle branches to the latissimus dorsi muscle arising in the "costal groove" segment of the three lowermost intercostal vessels (9^{th} to 11^{th} interspaces) were identified bilaterally in 28 fixed hemithoraces (84 interspaces). In the interspaces, the perforators > 0.5 mm were localized.

The study showed that in all three interspaces, at least one big perforator vessel was found to enter the undersurface of the latissimus dorsi muscle. Therefore it is possible to furnish two separate muscle, musculocutaneous, or even perforator flaps out of one latissimus dorsi muscle.

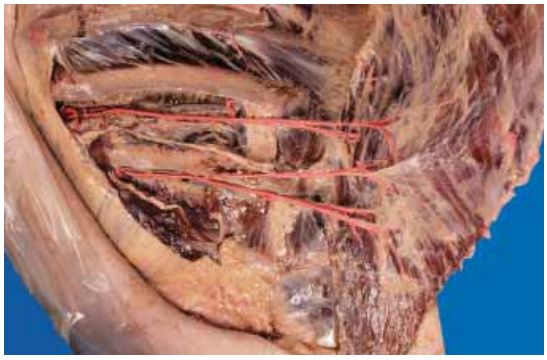


Figure 1
Intercostal vessels from the right costal groove segment of the 10^{th} and 11^{th} interspace with muscular branches to the undersurface of the latissimus dorsi. Note the rich branching of the vessels before the entrance to the muscle. When the pedicle of the intercostal vessels proves too short, additional length can be gained by just raising the paraspinous muscles and dissecting the intercostal vessels for another 5 to 6 cm medially.

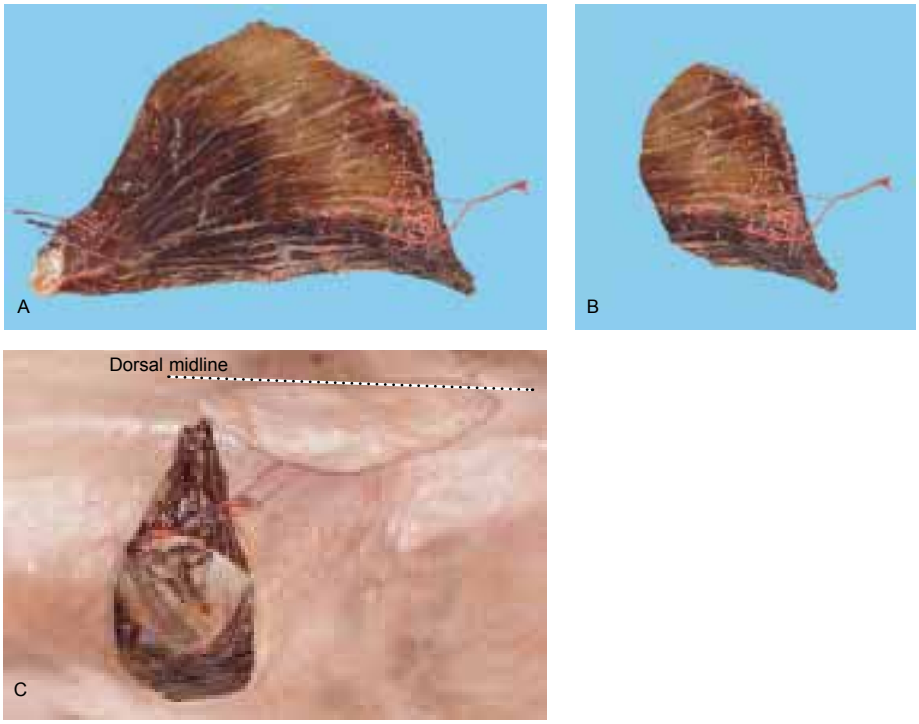


Figure 2
Variations of the latissimus dorsi muscle flap, A) bipedicled latissimus dorsi free flap with the thoracodorsal vessels on the left and one the intercostal vessels on the right, B) thin, "minor" latissimus dorsi free flap, pedicled on muscular brances of the intercostal vessels of the 10^{th} interspace, C) free style intercostal artery perforator flap centered over the 11^{th} intercostal space of the left hemithorax transposed over parts of the lumbosacral midline. Note the long pedicle with the wide arc of rotation.

Achievements 2004

- Identification of the thoracoacromial vessels as new recipient vessels for microsurgery.
- New identification of the latissimus dorsi flap as a bipedicled muscle flap.
- Identification of a new intercostal artery latissimus dorsi perforator flap.

Collaborations:

- Dr. H. Cristina-Schmitz, Institute for Laboratory Animal Science, Central Biological Laboratory, University of Zürich
- Dr. M. Manestar, A. Lang, Institute for Anatomy, University Zürich-Irchel
- Devision of Plastic, Aesthetic and Reconstruktive Surgery, Landesklinikum Feldkirch, Austria
- Clinic of Dermatology, University Hospital Zürich

Selected references:

- Beer GM, Burg D, Zehnder A, Seifert B, Steurer M, Grimaldi H, Meyer VE. (2004) Functional, electrophysiological and morphometric evaluation of nerve regeneration from coaptation on regenerated nerve fibres - an experimental study in the rabbit. J Reconstr Microsurg, 20 (2) : 159 - 166
- Meier, S.A., Lang, A., Beer G.M. (2004) Training von Mikroanastomosen and Polyurethangefässen zur Reduktion von Tierversuchen. ALTEX 21(3): 135 - 138
- Kompatscher P, Manestar M, Schuster A, Lang A, Seifert B, Beer GM (2005) The thoracoacromial vessels as recipient vessels in microsurgery and supermicro-surgery: a sonographic and anatomic study. Plast Reconstr Surg, 115 (1) : 77 - 83
- Beer GM, Lang A, Manestar M (2004) The bipedicled and the bipartite latissumus dorsie flap. Plast Reconstr Surg, in review
- Beer GM, Baumüller S, Zech N, Wyss P, Mihic-Probst D (2004) Immunohistochemic differentiation and site analysis of sweat glands in the axilla of normhidrotic patients. Plast Reconstr Surg, in press

2.4.2 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.

Differences in growth rate and differentiation in human chondrocytes cultured in the commonly used media
Dr. V. Wedler, Dr. M. Schneller, M. Welti

A culture media range in complexity from the relatively simple media containing essential amino acids, vitamins and salts, whereas a complex media and different serum free preparations contains a larger number of amino acids including nonessential amino acids, additional vitamins and are supplemented with minerals and extra metabolites (nucleosides, tricarboxylic acid, cyclic intermediates and lipids) in different combinations. We have compared constructs after culture in the three commonly used media for chondrocytes during dynamical culture on a biodegradable scaffold. The constructs have been assessed by DMMB assay, MTT, Collagen Type 2 and histology.

Differences in growth rate and differentiation in human chondrocytes in different concentrations of human serum
Dr. V. Wedler, Dr. M. Schneller, M. Welti

Tissue engineering in vivo most certainly requires the handling of isolated autologous cells where all steps has to be carefully executed in order to prevent immune response, avoid potential infections or contamination by media and supplements. This of course excludes the use of bovine sera. Ideally should autologous sera be used. The first clinical attempts with autologous chondrocyte transplantation were presented by Brittberg et al. He and other authors have used autologous serum in concentrations from 10 to 20%. These relatively high serum concentrations are hardly not possible when chondrocytes are being cultured in a bioreactor on a scaffold, since the amount of needed autologous serum is limited. We have compared constructs after culture in 5 and 10% human serum during dynamical culture on a biodegradable scaffold. The constructs have been assessed by DMMB assay, MTT, Collagen Type 2 and histology.

Sequential supplementation with TGF-ß1, IGF-1 and BMP-2 in human articular chondrocytes
Dr Maria Schneller-Gustafsson, MD; Pascal Herzog; Dr Volker Wedler, MD

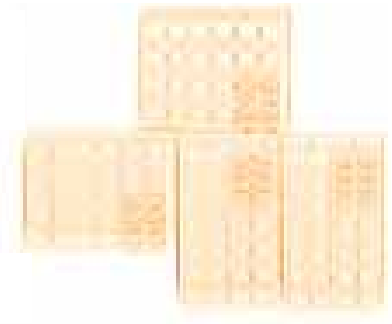
Tissue engineering of cartilage consists of two steps. First, autologous cells have to be multiplied. During this process the cells lose their cartilage phenotype. Secondly, these cells have to be stimulated to redifferentiate and produce cartilage matrix. Different growth factors can be used to increase cell proliferation, redifferentiation, extracellular matrix production and maturation. Additionally, it is believed that in order to obtain a qualitatively superior extracellular matrix, not only must the correct growth factors be given, they must also be administered in an optimal sequence.

In cooperation with:
· 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.



Anti-aging therapy in tissue engineering: An approach in growing human chondrocytes

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

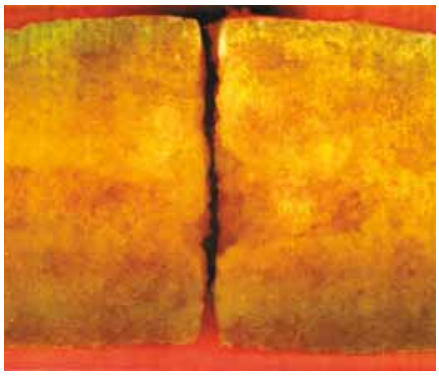
The advancement in medicine requires an ever-faster provision of biological tissue substitutes. This also concerns the tissue engineering of cartilage cells. In order to provide patients and physicians as fast as possible with autologous substitutes in the case of degenerative illnesses or after trauma, 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

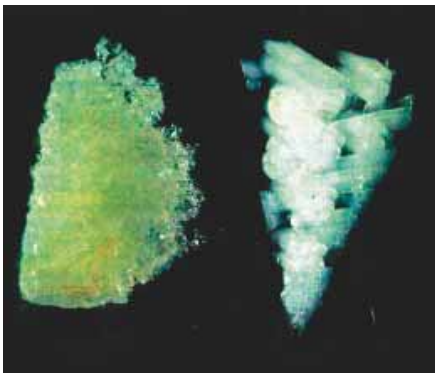
Anti Aging therapy becomes increasingly important in many disciplines of medicine. It is an old method in a trendy position. There are some other terms such as better aging, rejuvenation or age medicine that all refer to the same concept. But who works in this new field of hormones, diets and sports 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 (18^{th} and 19^{th} 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.

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.



Scaffold (Innovent®/ Germany): 14 days after cultivation with human chondrocytes



Two different types of scaffolds (left: Innovent®/ Germany, right: Isotis®/ Netherland)

Collaborations:

- Dr. J.P. Hellermann, Departement für Kardiologie, Epidemiologie, USZ
- Prof. Dr. Th. Bächi Elektronenmikroskopisches Zentrum Zürich
- Klaus Marquard, Elektronenmikroskopisches Zentrum Zürich
- Dr. B. Bode, Departement Klinische Pathologie, USZ
- Prof. Dr. von Rechenberg, Tierspital Zürich
- Dr. C. Rohrer, Onko Service Tierspital Zürich
- Dr. D. Cantieni, Tierarztpraxis TAU, Horgen
- Dr. N. Zech, Departement für Gynäkologie, USZ
- Dr. J. Roos, Departement für Radiologie, USZ
- Dr. P. Neuenschwander, ETH Hönggerberg, Biomaterials
- Dr. L. Moroni, ISOTIS Holland
- Dr. M. Schnabelrauch, INNOVENT Jena
- PD Dr. D. Weishaupt, MR Zentrum, USZ

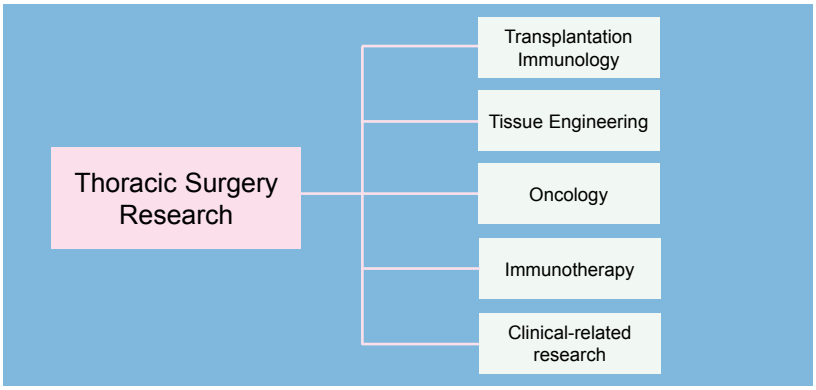
Selected references:

- 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 (in review)
  C. Koehler, D. Weishaupt, W. Kuenzi, V. Wedler
- Extensive Hydrofluoric Acid Injuries: A Serious Problem
  Wedler, Volker MD; Guggenheim, Merlin MD; Moron, Miguel MD; Künzi, Walter MD; Meyer, Viktor Emanuel MD
  J Trauma Volume 58(4) April 2005 pp 852-857 (Accepted: 12. Nov. 2003)

2.5 Thoracic Surgery Research

PD Dr. med.
Stephan
Korom

Prof. Dr. med.
Walter Weder



2.5.1 Transplantation Immunology

PD Dr. med.
Stephan Korom

Dr. med.
Markus Cardell

Dr. med.
Florian Jung

Dr. med.
Wei Zhai

Melatonin and the allogeneic challenge: observations following perfused organ transplantation
M. Cardell, MD, Dr. med. F. Jung, Dr. med. W. Zhai, PD Dr. med. S. Korom

Melatonin (MLT), secreted by the pineal gland, is a multifunctional neurohormone which (a) protects tissues from damage through free radicals and attenuates ischemia/reperfusion injury in organ grafts; (b) acts synergistically on cellular antioxidants; (c) displays a complex, dose-dependent immunoenhancing and -suppressing effect in vitro and -vivo. Our research is focussed on the organoprotective (anti-ischemia/reperfusion injury) and potential immunosuppressive effects of MLT therapy.
Recently, we have shown for the first time that high-dose MLT therapy abrogates acute allograft rejection, effecting humoral and cellular rejection pathways. These findings may indicate a novel therapeutic immunosuppressive approach, based on modulation of the neuroendocrine/immune axis. Further studies are under way to decipher this intricate network between the brain and the immune response, triggered by exposure to allogeneic antigen.

Specific inhibition of CD26/DPP IV enzymatic activity: novel investigations and inhibitors
M. Cardell, MD, Dr. med. F. Jung, Dr. med. W. Zhai, PD Dr. med. S. Korom

The lymphocyte surface glycoprotein CD26 acts as T cell costimulator, its dipeptidyl peptidase (DPP IV) catalytic activity is linked to its costimulatory efficacy in vitro and to immunocompetence in vivo. We investigate the effect of an irreversible DPP-IV inhibitor on the course of acute rejection in a model of orthotopic single lung transplantation in the rat. We have shown for the first time that a Pro-Pro-diphenyl phosphonate derivative abrogates the immune cascade leading to acute pulmonary rejection, maintains pulmonary function and preserves histomorphological architecture. These results extend earlier findings and stress the role of CD26/DPP IV in alloantigen-mediated T-cell responses.

Currently, novel inhibitors and investigational approaches in the field of transplantation immunity are being assessed.

Achievements 2004

- Presentations:
  FJ Jung, L Yang, L Härter, I Inci, D Schneiter, D Lardinois, M Keel,W Weder, S Korom. Melatonin and acute cardiac allograft rejection.
  Society of Heart and Lung Transplantation, 24.04.2004, San Francisco, USA.

  FJ Jung, L Yang, I De Meester, K Augustyns, S Hillinger, D Lardinois, P Vogt, S Scharpe, W Weder, S Korom. CD26/Dipeptidyl peptidase IV-targeted therapy of acute lung rejection in rats. American Society of Transplantation, 14.-19.05.2004, Boston, USA.

  FJ Jung, L Yang, L Härter, D Schneiter, D Lardinois, M Keel, W Weder, S Korom. Melatonin abrogates acute and accelerated cardiac rejection in rats. FJ Jung, L Yang, L Härter, D Schneiter, D Lardinois, M Keel, W Weder, S Korom. American Society of Transplantation, 14.-19.05.2004, Boston, USA

  Procedures performed:
  45 lung transplantation (LTx) in rats, 40 heart transplantation (HTx) in rats, 110 lung explantation/backtable (heart beating and non-heart-beating), 10 single orthotopic lung transplantations in pigs

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).
- Korom S, De Meester I, Stadlbauer THW, et al. 1997. Inhibition of CD26/dipeptidyl peptidase IV activity in vivo prolongs cardiac allograft survival in rat recipients. Transplantation 63:1495-1500.
- De Meester I, Korom S, Van Damme J, and Scharpé S. 1999. CD26, let it cut or cut it down. Immunology Today 20: 367-375.

2.5.2 Tissue Engineering

Dr. med.
Lin Yang

Dr. phil.
Maurus Curti

Dr. med.
Qiang Tan

Manfred Welti

Tissue engineering of the trachea: novel techniques, cells and scaffolds
Dr. phil. M. Curti, M. Welti, Dr. med. Q. Tan, PD Dr. med. S. Korom

In the previous project, cartilage-like tissue was successfully designed using chondrocytes, harvested from human tracheal cartilage and seeded on a novel elastic, degradable polyurethane polymer (DegraPol®-foam). However, the long-term aim of our SNF supported studies is tracheal replacement with a functioning artificially engineered neo-trachea. To move closer to this goal, we focussed on two major issues in 2004:

- Investigation of new kinds of scaffolds
- Improvement of seeding techniques

Investigation of a new kind of scaffold with a new seeding strategy
In addition to Dr. Neuenschwander from the ETH, our hitherto supplier of scaffolds, we started a new collaboration with L. Moroni from Isotis® (Netherlands). They provided us with a selection of polyethyleneoxide-terephthalate (PEOT) and polybutylene-terephthalate (PBT) (Polyactive®) scaffolds.
Using both materials, a soft/amorphous part (PEOT) is combined with a hard/crystalline (PBT) structure, rendering them both biocompatible and biodegradable. We tested four different types of Polyacive™ scaffolds VT1, VT2, C1 and C2 with different specifications each in a composition of 55% PEOT and 45% PBT (Tab. 1, Fig. 4).

	VT1	VT2	C1	C2
Outer Ø [mm]	7.5	7.5	10	10
Inner Ø [mm]	5	5	5	5
Fiber Ø [µm]	170	170	170	170
Fiber spacing [µm]	600	400	600	600 & 800 alternated
Layer thickness [µm]	150	150	150	150
Porosity [%]	75	62	75	75-81

Table 1. 3D specifications of the Polyactive™ scaffold tubes

Figure 4. The four variants (from left to right: VT1, VT2, C1, C2) of Polyactive™ scaffold tubes.

Two samples of each scaffold type were seeded for three days in a chondrocyte suspension at a concentration of 1×10^6 cells/ml. After this seeding period, the scaffolds were extracted from the chondrocyte suspension, gently washed in cell free medium (Ham's F12 supplemented with 10% foetal bovine serum and Penicillin / Streptomycin), and cultured in 75cm^2 Rectangular Canted Neck Cell Culture Flasks with Vent Cap (Costar®) for three weeks.
To prevent sedimentation of the chondrocytes during the seeding period, it is necessary to apply a dynamic procedure. Apart form the conventional method to keep the cells in suspension by means of a spinner flask we tested a novel multi-rotator device (MACSmix®) (Fig. 5).

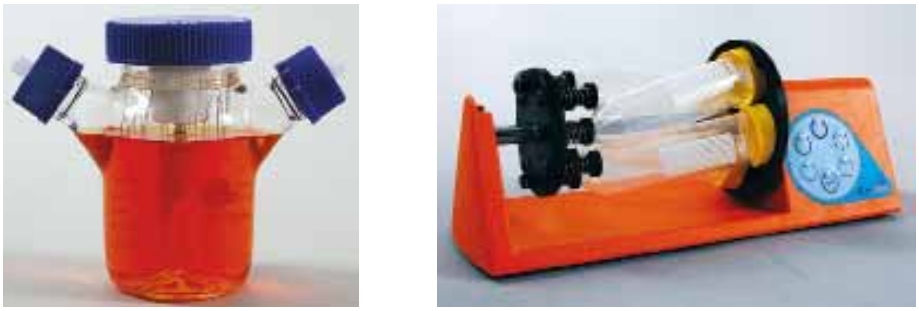


Figure 5. Spinner flask (left) and MACSmix®. The MACSmix® device is a versatile tube rotator suitable for a temperature range of 2 °C to 42 °C. Thus it can be placed in an refrigerator or incubator, and can be equipped with tubes from 0.5 ml to 50 ml in size. The device operates at three different speeds or at set intervals.

Figure 6. TPP tissue culture container with filter cap

To allow for gas exchange we used special tissue culture containers (TPP) (Fig 6).

Culture growth occurs using suitable supports while rotating at a temperature of 37°C. The gas-porous filter guarantees oxygen supply, even at very high tissue densities. Regulated gas transfer will satisfy every requirement. Sterility is maintained at all times by means of the additional filter membrane (0.22µm). The efficacy of the seeding was tested by MTT and GAG assay, histology and by scanning electron microscopy (SEM). The MTT assay is a colorimetric method to quantify the number of viable cells by their capacity to reduce the tetrazolium salt MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) by the mitochondrial succinate dehydrogenase. This reduction results in the formation of a water-insoluble formazan salt, which is subsequently solubilized in 20% SDS in 50% dimethyl formamide (pH 4.7). The GAG assay is also a colorimetric method to quantify glycosaminoglycan, the major component of the extracellular matrix (ECM) of cartilage.
According to the MTT data, all the scaffolds that were seeded with the MACSmix™ device showed a higher load of cells per unit of weight than those seeded by means of a spinner flask. Moreover, the scaffolds seeded with the MACSmix™ device also showed more extracellular matrix glycosaminoglycan per unit of weight than those scaffolds seeded in the spinner flask. This difference in MTT and GAG values according to the seeding method was most pronounced in the scaffolds with the VT1 specifications (Fig. 7&8).

Scanning electron microscopy confirmed the result, that cartilage formation on the scaffolds failed upon seeding them in a spinner flask. The cells merely adhered at few areas on the scaffolds, whereas the major part of the surface of the scaffold remained free of cells. In contrary, after seeding the scaffolds by using the MACSmix™ device, large parts of the scaffold were covered with chondrocytes and newly synthesized ECM (Fig. 9).

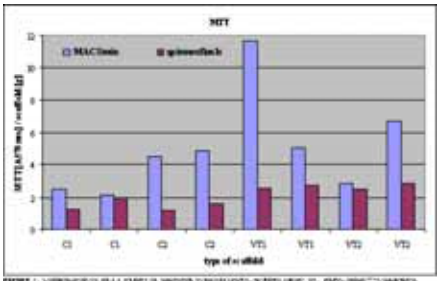


Figure 7. Comparison of MTT values of different scaffold types, spinner flask- vs. MACSmix™-cultivated.

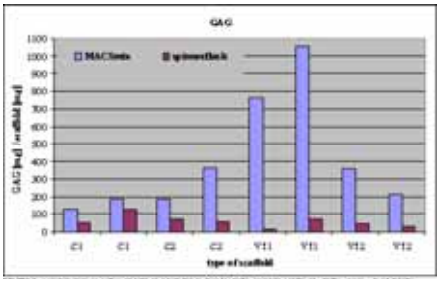


Figure 8. Comparison of MTT values of different scaffold types, spinner flask- vs. MACSmix™-cultivated.

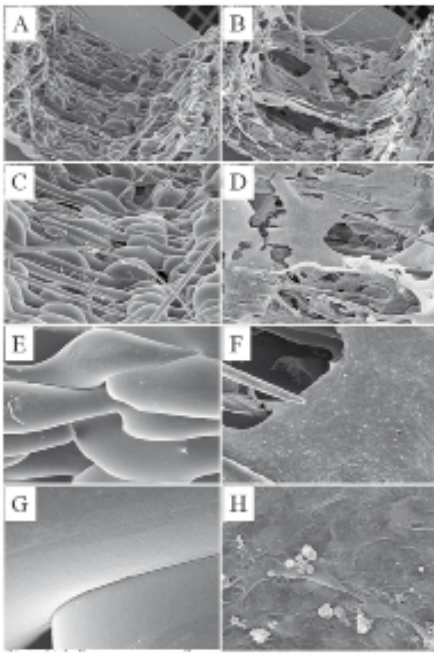


Figure 9. Scanning electron microscopy: A&B 10 × magnification, C&D 20 × magnification, E&F 65 × magnification, G&H 650 × magnification; A,C,E,G seeded in spinner flask B,D,F,H seeded in MACSmix®

Achievements 2004

- Implementing a novel dynamic seeding technique for ISOTIS® scaffolds.

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.

Selected references:

- Yang L, Korom S, Welti M, Hoerstrup SP, Zund G, Jung FJ, Neuenschwander P, Weder W. 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.
Didier Lardinois

Dr. med.
Isabelle Opitz

Pharmacokinetic Study After Intrapleural Topical Application Of Chemotherapeutic Agents For Malignant Pleuromesothelioma In Immune Competent Rat Model

PD Dr. med. Didier Lardinois

Objective: To investigate if local intrapleural application of cisplatin with the aid of a surgical sealant has a prolonged local pharmacological tissue level in comparison to application of cisplatin solution while reducing systemic drug exposure.
Methods: Forty immune competent Fischer rats (300g ± 32g) were inoculated with a syngeneic malignant mesothelioma cell line (II-45). 300µl (10^6 cells) were injected in the left chest cavity. Ten days later, left pneumonectomy with tumor debulking and mechanical abrasio was performed. The animals were then equally divided in two groups without any selection. Twenty animals received local application of cisplatin solution (100 mg/m^2) whereas cisplatin was topically applicated with a gel of surgical sealant (Vivostatâ, Vivolution, Denmark) at the same concentration in 20 other animals. In each group, 5 subgroups of 4 animals each were defined. Successive plasma and tissue (pleura) samples were collected at 2 hours, 4 hours, 1 day, 3 days, and 1 week after local therapy. Platinum concentrations in plasma and tissue were assessed by a physician blinded to the study subgroups by use of inductively coupled plasma mass spectrometry detection. To avoid bias, two plasma samples, respectively three samples of tissue were harvested at each time in each animal and analyzed. Urea nitrogen and creatinin levels were determined in plasma to evaluate systemic renal toxicity of platinum. Statistical analysis was performed by use of the two-sample t-test.
Results: Platinum concentrations in tissue were significantly higher in the group cisplatin + Vivostat® gel than in the group cisplatin solution at 1, 3, and 7 days after therapy ($p = 0.007$, $p = 0.005$, and $p = 0.0002$, figure 1). There was no statistically significant difference in platinum concentrations in plasma at any time between the two groups. Comparison of urea nitrogen and creatinin before harvesting showed a renal insufficiency in the animals of the group cisplatin solution at 1 week after therapy with values of 98 mmol/l vs. 7.7 mmol/l for urea nitrogen and of 410 µmol/l vs. 43 µmol/l for creatinin ($p = 0.02$ and $p = 0.05$).
Conclusions: Intrapleural topical administration of cisplatin in combination with surgical sealant Vivostat® provides significantly sustained higher platinum concentrations in tissue in comparison to application of cisplatin solution without conferring systemic toxicity in this rat model. The concept of this experimental project might improve local control of the disease in a multimodality therapy strategy of malignant pleuromesothelioma.

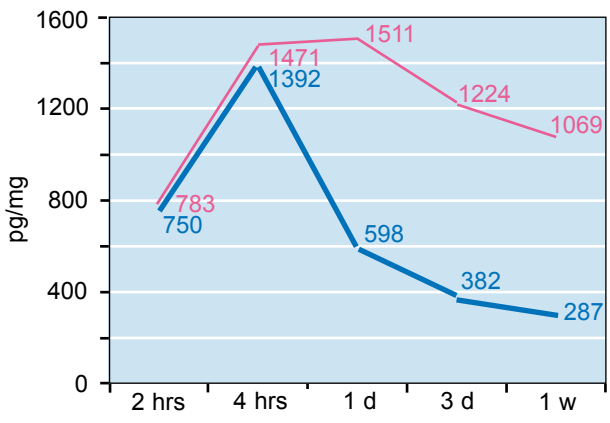


Figure 1: Platinum concentrations in tissue (pleura) according to the type of administration (solution vs. gel) at different time after intrapleural application

Achievements 2004

- Intrapleural topical administration of cisplatin in combination with surgical sealant Vivostat® provides significantly sustained higher platinum tissue concentration in an immune competent rat model of malignant pleural mesothelioma - manuscript submitted

Collaborations:

- Thoracic Surgery (F. Jung, I. Opitz, W. Weder)
- Radio-Onkologie (V. Vuong)
- Onkologie (R. Stahel)
- Pathologie (P. Vogt)
- Chemische Institut (K. Rentsch, C. Latkoczy)
- Biostatistics (V. Rousson)

Selected references:

- Ratto GB, Civalleri D, Esposito M, Vannozzi MO. Pleural space perfusion with cisplatin in the multimodality treatment of malignant mesothelioma: a feasibility and pharmacokinetic study.
  J Thorac Cardio-vasc Surg 1999; 117:759-65.
- Prewitt et al. Orthotopic implantation of mesothelioma in the pneumonectomized immune-deficient rat: a model for innovative therapies.
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- Hanada K, Ninomiya K, Ogata H. Pharmacokinetics and toxicodyna mics of cisplatin and its metabolites in rats: relationship between renal handling and nephrotoxicity of cisplatin.
  J Pharm Pharmacol 2000; 52: 1345-53

Malignant pleural mesothelioma - new intrapleural adjuvant strategies during pleuropneumonectomy - in vitro study

Dr. med. I. Opitz, Dr. med. S. Hillinger, Dr. phil. M. Curti, PD Dr. med. D. Lardinois

Background: The poor prognosis of malignant pleural mesothelioma with a median survival of 9 months for untreated patients is due to a locally aggressive growth pattern. Even with multimodal treatment strategies including extrapleural pneumonectomy combined with chemotherapy and radiotherapy, local recurrence is common. [1] Intrapleural adjuvant therapy regimes are an attractive treatment option for local tumor control. The adjuvant use of intrapleural taurolidin, a synthetic broad-spectrum antibiotic with antiproliferative activity on gastrointestinal tumors [2] and povidone-iodine with proven cytotoxic effect on colon cancer cells [3], was evaluated in an in-vitro pilot study.

Materials and Methods: Two human mesothelioma cell lines (ZL5 and ZL55) cultivated from tumor biopsy after pleuropneumonectomy and pleural effusion of malignant mesothelioma patients as well as a rat malignant mesothelioma cell (II-45) established from experimentally induced meostheliolmas in Fisher 344 rats exposed to asbestos. Cell growth was measured by use of the colorimetric MTT-assay. Taurolidine 2% was added in a volume of 50 µl to achieve a final concentration ranging from 0-1%. Braunol® (=povidone-iodine) was added in a concentration of 0-0.2%. Control wells received 50 µl of medium. Cells were exposed for 7.5 min, 15 min, 30 min, 1 hour, 2 hours, 24 and 72 hours to both substances. Substrate cleavage was monitored at 570 nm by a Synergy HT (Biotek, Vermont, USA) microplate reader and analyzed using the KO4 software 3.4 Microplate Data Analysis. Each sample was performed in triplicate.

Results: Taurolidine 2% and povidone-iodine showed influence on cell growth of the human cell line (epithelial and mixed type). A similar effect could be observed for the rat cell line. Independently of the concentration used, 24 hours after incubation with Taurolidine, all cells were dead. Povidone-iodine directly killed the cells but already after 7.5 min exposure time from a concentration of 0.02% onwards.

Conclusion: The cytotoxic influence of Povidone-iodine and Taurolidine 2% may be an adjuvant therapy option during pleuropneumonectomy for malignant pleural mesothelioma. Outlook: In vivo studies in a rat mesothelioma model are planned. In a first step a new recurrence model will be established in the rat. Subsequently the impact of intrapleural adjuvant therapy with Taurolidine or povidone-iodine or CCL-19 - an immunomodulating cytokine - on the recurrence rate after pleuropneumonectomy will be analyzed in a randomized design.

Achievements 2004

- Establishment of experimental assays, animal licence application for in vivo studies submitted

Collaborations:

- Department of Oncology (Sally Donaldson, Rolf Stahel)

Selected references:

- Rusch V., Niedzwiecki D., Tao Y., Markman M. Intrapleural cisplatin and mitomycin for malignant mesothelioma following pleurectomy: pharmaco-kinetic studies. J Clin Oncol 1992; 10: 1001 6.
- Opitz I, Van Der Veen HC, Braumann C, Ablassmaier B, Fuhrer K, Jacobi CA. The influence of adhesion prophylactic substances and taurolidine/heparin on local recurrence and intraperitoneal tumor growth after laparoscopic-assisted bowel resection of colon carcinoma in a rat model. Surg Endosc. 2003 Jul;17(7):1098-104. Epub 2003 Apr 28.
- 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

2.5.4 Immunotherapy for lung cancer

Dr. med.
Sven Hillinger

Dr. med.
Markus Cardell

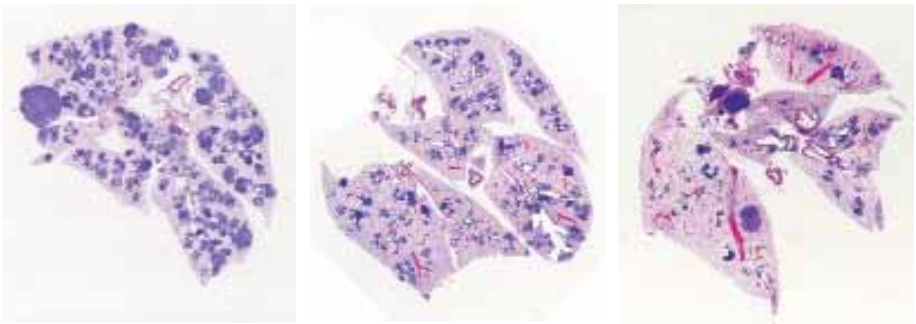
Dr. phil.
Maurus Curti

Epstein Barr virus-induced molecule 1 ligand chemokine (ELC/CCL19) in lung cancer therapy

Dr. med. Sven Hillinger

Effective anti-tumor responses require both antigen presenting cells (APC) 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. In addition, tumor cells produce inhibitory factors that promote escape from immune surveillance. Consequently, effective anti-cancer immunity may be achieved by recruiting professional host APC for tumor Ag presentation to promote specific T cell activation. Based on the capacity of ELC/CCL19, a CC chemokine expressed in T cell zones of spleens and lymph nodes, to attract both naïve T cells and mature dendritic cells (DC) we have shown that intratumoral ELC/CCL19 administration reverses tumor-mediated immune suppression and orchestrate effective cell-mediated immune responses by establishing a chemotactic gradient that favors localization of DC within the tumor site (Ref.). The transgenic CC-10 Tag mice, in which the SV40 large Tag is expressed under control of the murine Clara cell-specific promotor, adenocarcinomas develop in an organ-specific manner and, compared with transplantable tumors, the pulmonary tumors in these mice more closely resemble human lung cancer. Mice expressing the transgene develop diffuse bilateral bronchoalveolar carcinoma and have an average life span of 4 months. We evaluated the antitumor properties of ELC/CCL19 in this spontaneous murine model of lung cancer. Histological evaluation of tumor sections from mice treated at three months of age with recombinant ELC/CCL19 (0.5mg/dose) by intra-peritoneal or intranodal (axillar lymph node region) injection three times per week for 4 weeks revealed extensive lymphocytic infiltration with a marked reduction in tumor burden compared to diluent treated 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 lungs of ELC/CCL19 treated mice. Lung tissue cytokine profiles showed a shift towards immunostimulatory molecules.

Lungs of 4 months old CC10 transgenic mice



Control

CCL19-treated intraperitoneal for 4 weeks, 3x/week

CCL19-treated intranodal for 4 weeks, 3x/week

Furthermore we have been successful in establishing an orthotopic model of lung cancer in mice which we can treat loco-regional and which also mimics the clinical situation more closely than the widespread subcutaneous models.

Future projects will address additional possibilities of this therapy in combination with other preventive or therapeutic options. With these experiments we will clarify the effectiveness and mechanisms of a novel immunotherapeutic strategy, which would be highly feasible for clinical application in all lung cancer patients.

In 2004 we have been able to acquire additional lab space, which gives us the ability to separate sterile bench and cell culture work from molecular and biological work and also take into account the additional lab members.

Achievements 2004

- Zürich Cancer League and Sassella-Stiftung: 'Epstein Barr virus-induced molecule 1 ligand chemokine (ELC/CCL19) in lung cancer therapy'

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 Anti-tumor Responses in a Lung Cancer Model.
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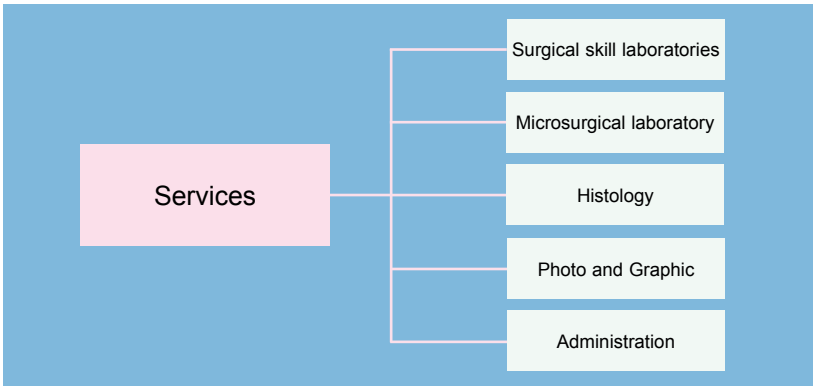
- Yang SC, Hillinger S, Riedl K, Zhang L, Zhu L, Huang M, Atianzar K, Kuo BY, Gardner B, Batra RK, Strieter RM, Dubinett SM, Sharma S.
  Intratumoral administration of dendritic cells overexpressing CCL21 gene rates systemic antitumor responses and confers tumor immunity.
  Clin Cancer Res. Apr 15;10 (8):2891-901 (2004)

3. Services




Boris Leskosek


Alush Avdyli

3.1 Surgical skill laboratories

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


Vlasta Strohmeier

3.2 Microsurgical laboratory

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


Astrid Morger

3.3 Histology

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

3.4 Photo and graphic services

Nico Wick,
Photographer

Lea Schütz-Cohen,
Photographer

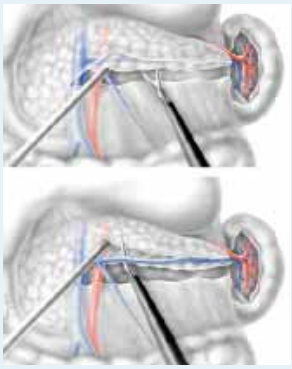
Stefan Schwyter,
Scientific
Illustrator

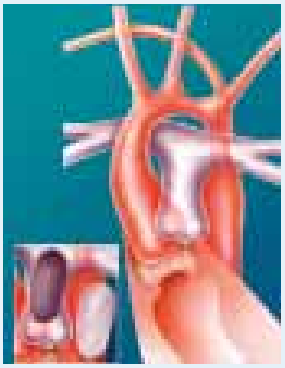
Carol De Simio,
Scientific
Illustrator

A quick, flexible, versatile and professional service.

We offer

- photographic documentation of patients
- tecnical photography, on location or in our well equipped studio
- reproductions from any original
- slide exposing from any file
- laserprint (up to A4) and inkjet (up to A3) on any material
- preparing of files for external printing
- layout of printing matters
- graphic and design of illustrations for papers and books
- construction and maintainance of websites
- maintainance of the digital image archives





Gabriella Muolo-
Giaquinta,
Administration
Division of
Surgical Research

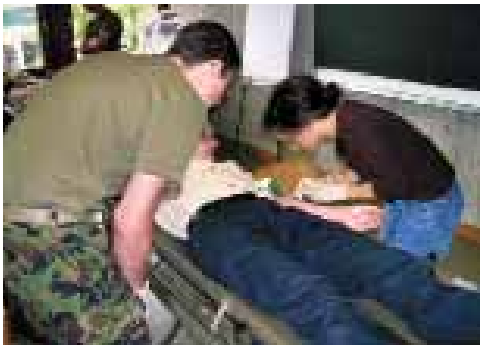
3. 5 Administration

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

4. Events and Workshops at the Surgical Research Division in 2004

New Surgical Skill Laboratory

New Microsurgical Laboratory



Student stiching and injection classes

Farewell Party Dr. O.A. Trentz

5. Publications 2004

- Beer GM, Burg D, Zehnder A, Seifert B, Steurer M, Grimaldi H, Meyer VE. Functional, electrophysiologic, and morphometric evaluation of nerve regeneration from coaptation on regenerated nerve fibers: experimental study in rabbits. J Reconstr Microsurg 2004;20:159-66
- Bimmler D, Schiesser M, Perren A, Scheele G, Angst E, Meili S, Ammann R, Graf R. Coordinate regulation of PSP/reg and PAP isoforms as a family of secretory stress proteins in an animal model of chronic pancreatitis. Journal of Surgical Research 2004;118:122-35.
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- Bosshart H, Heinzelmann M. Human neutrophil-derived CAP37 inhibits lipopolysaccharide-induced activation in murine peritoneal macrophages. Immunol Lett 2004;94:175-82.
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- Grunenfelder J, Umbehr M, Plass A, Bestmann L, Maly FE, Zund G, Turina M. Genetic polymorphisms of apolipoprotein E4 and tumor necrosis factor beta as predisposing factors for increased inflammatory cytokines after cardio- pulmonary bypass. J Thorac Cardiovasc Surg 2004;128:92-7.
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- Mica L, Härter L, Trentz O, Keel M. Endotoxin reduces CD95-induced neutrophil apoptosis by cIAP-2-mediated caspase-3 degradation. J Am Coll Surg 2004;199:595-602.
- Mol A, Bouten CV, Baaijens FP, Zund G, Turina MI, Hoerstrup SP. Review article: Tissue engineering of semilunar heart valves: current status and future developments. J Heart Valve Dis 2004;13:272-80.
- Mol A, Hoerstrup SP, Heart Valve Tissue Engineering- Where do we stand? Int J Cardiol 2004;95 Suppl 1:57-58.
- Neuenschwander S, Hoerstrup SP. Heart valve tissue engineering. Transpl Immunol 2004;12:359-65.

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6. Grants 2004

Cardiac Surgery

Grants	Title of Project	Project Leader
Federal Comission for Technology and Innovation (CTI/KTI)	Herstellung Tissue Engineering für Patienten mit angeborenen Herzfehlern	Prof. Zünd Prof. Hoerstrup
SNF (NF46)	Endothelial cells 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

Visceral & Transplant Surgery

Grants	Title of Project	Project Leader
Hepatobiliary laboratory		
SNF	Soluble mediators and cellular receptors in the ischemic liver	Prof. Clavien
NIH	Mechanisms of endothelial cell death in the ischemic 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
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
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
UBS	Veränderte Expression von Tyrosin-/MAP-Kinasen und Kaspasen nach Schock und während der Sepsis als neuer pathophysiologischer Mechanismus des inflammatorischen Multiorganversagens - Regulation und Modulation"	Dr. Ertel, Dr. Keel
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 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
Olga Mayenfisch - Melatonin (MLT)	Melatonin in der Transplantationsimmunologie	PD Dr. Korom
Olga Mayenfisch - Tissue Engineering (TE)	Tissure-engineering zur Trachealrekonstruktion	Dr. Yang
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

7. Awards 2004

- G. Zünd, S.P. Hoerstrup: Pioneer Award 2004, Zürich State Bank, Switzerland
- D. Schmidt, S.P. Hoerstrup: Young Investigator Award/ Marko Turina Award, European Association of Cardiovascular Surgery
- N. Selzner received the young investigator award from the Gastroenterological Society (Geneva)
- D. Dindo received an award from the Swiss Society of Surgery

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