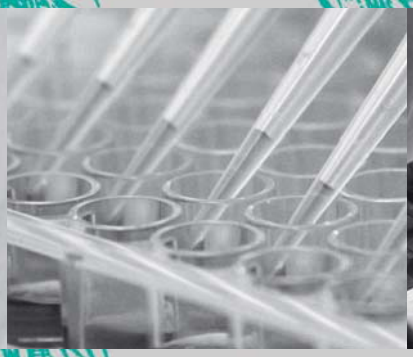
Division of Surgical Research

Annual Report 2006

Department of Surgery
University Hospital Zurich
Switzerland



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

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

Content

Preface

Dear Colleagues

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

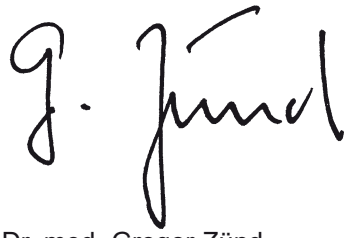
It is my privilege to present the Annual Report 2006 of the Division of Surgical Research at the Department of Surgery, University Hospital Zurich.

In the year 2006, a new administrative organisation was established which today is very much appreciated by all technicians and researchers. The Division's quarterly internal reports comprising the corresponding operational expenditures as well as the investments carried out allow for a quality control by means of timely financial project management. The investments performed in the past year included the acquisition of an oxymeter, a micro-surgery microscop, new sets of micro-surgery instruments and a small-animal anaesthesia-apparatus. All acquisitions are being used by various research groups within the Division complementing the apparatus pool accordingly.

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

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

Yours sincerely



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

1. Organisation

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

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

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

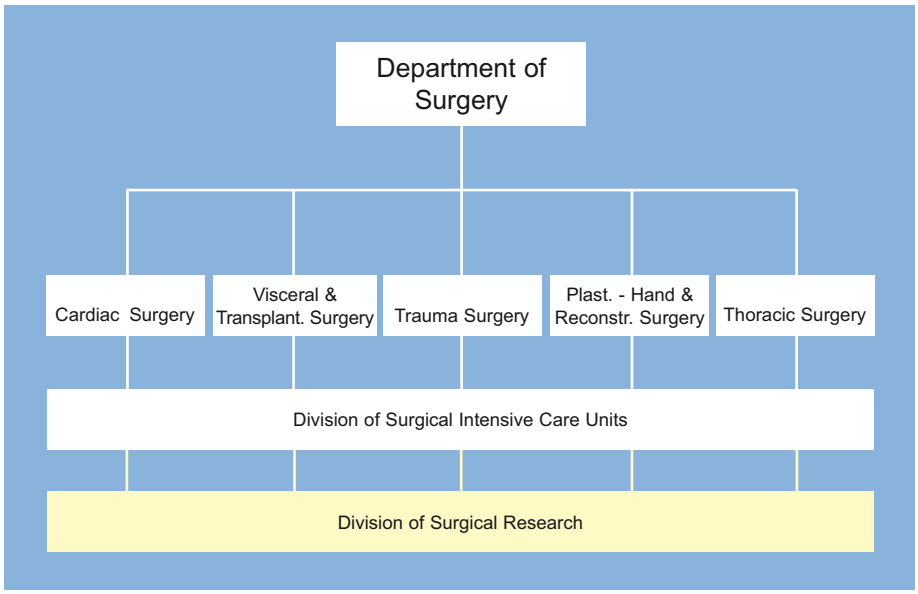
Prof. Dr. med.
Walter Weder,
Director Clinic
Thoracic Surgery

Prof. Dr. med.
Michele Genoni
Director Clinic
Cardiac Surgery

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

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

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

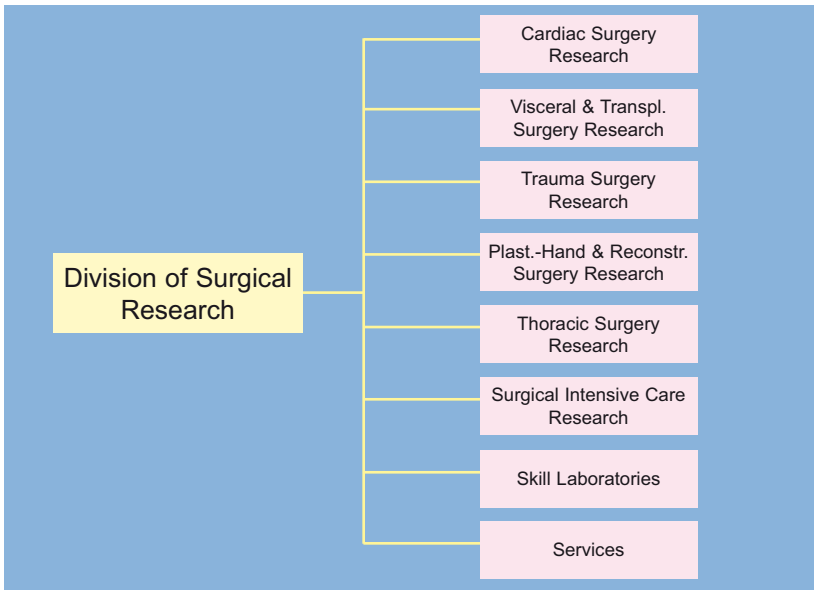


1.2 Structural Organisation of the Division of Surgical Research

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

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

Juliana Brink-Bogo,
Administration
Division of Surgical
Research



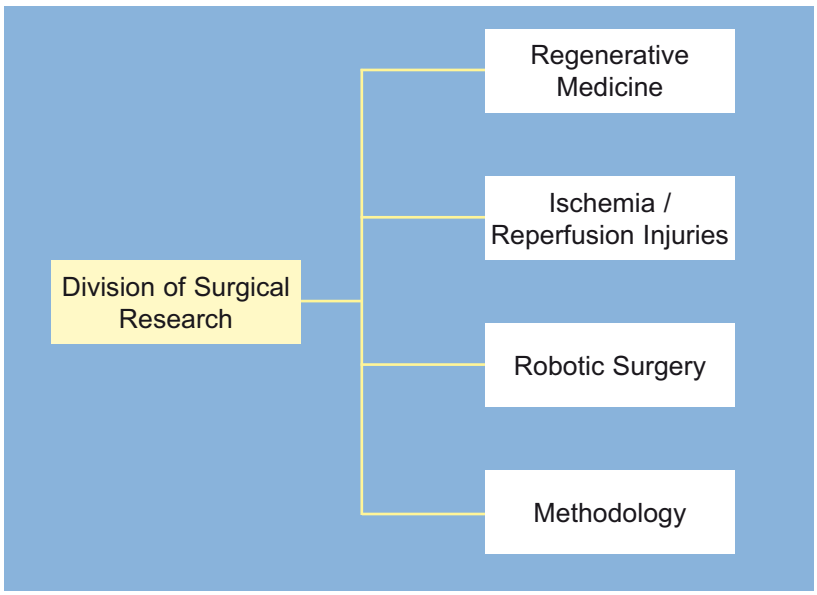
1.3 Scientific Sections within the Division of Surgical Research

Prof. Dr. med.
Simon Philipp
Hoerstrup
Regenerative
Medicine

Dr. phil II
Wolfgang Moritz
Ischemia /
Reperfusion
Injuries

PD Dr. med.
Peter Messmer,
Robotic Surgery

PD Dr. phil II
Rolf Graf
Methodology



2. Research and Development

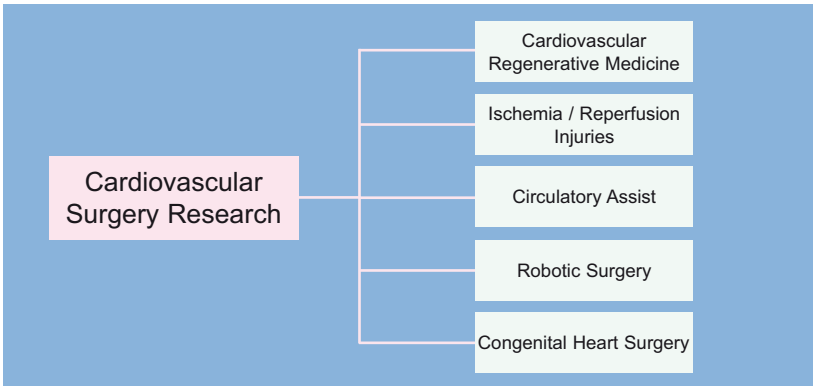
2.1 Cardiovascular Surgery Research


Prof. Dr. med.
Simon Philipp
Hoerstrup


Prof. Dr. med.
Gregor Zünd


Prof. Dr. med.
Michele Genoni


Sandra Edwin
Study Coordination
and Administration



2.1.1 Cardiovascular Regenerative Medicine (Tissue Engineering and Cell Transplantation)

Prof. Dr. med. Simon Philipp Hoerstrup


Prof. Dr. med.
Simon Philipp
Hoerstrup


Dr. med.
Dörthe Schmidt


Dr. sc. nat.
Jens Kelm


Dr. med.
Christian Schmidt


Rene Stenger
Bachelor
Chemistry


Anita Mol
PhD


Dr. med.
Alberto Weber


Prof. Dr.
Vijay Kumar


cand. med.
Armin Zürcher


cand. med.
Silvan Holdener


cand. med.
Sandro Imbach


cand. med.
Marek Balikowski


Praktikant
Volker Lorber

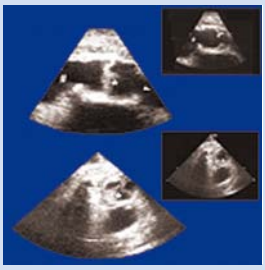
The Cardiovascular Regenerative Medicine Program comprises Tissue Engineering and Cell Transplantation and is focused on the development and in vitro generation of novel, cell based therapies for cardiovascular applications. These include tissue engineered blood vessels, heart valves as well as microscale strategies for myocardial regeneration. Presently utilized heart valve and blood vessel prostheses carry disadvantages for the patients mainly because non-living, artificial devices are inserted into the human organism. Tissue engineering enables the in vitro production of autologous, living and functional replacements with the capacity of growth for congenital application as an alternative to state of the art artificial replacements. Furthermore, an additional focus is the development of cell based implants based on the design of in vitro generated microtissues to improve myocardial functionality of the diseased heart.

Research projects:

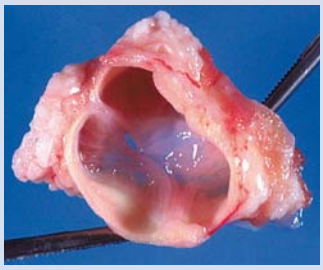
- Human Cell-Based Systems (progenitor, fetal, adult)
- Extracellular Matrix (proteins, tensegrity)
- Biomaterials (biodegradable, intelligent material systems)
- Bioreactor Systems
- Biomechanics, Computational Models, Molecular Imaging
- Animal Models (small and large)
- Tissue Engineered Cardiovascular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

Proof of Heart Valve Tissue Engineering Concept

pre-implantation



in-vivo

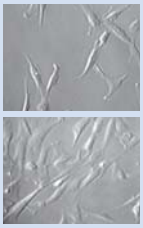


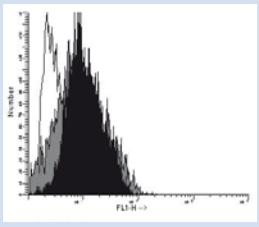
post-explantation

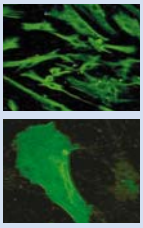
Autologous living tissue engineered heart valve in a sheep model, based on vascular - derived myofibroblasts and endothelial cells

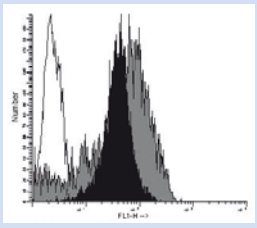
Hoerstrup et al. Circulation 2000

Human Heart Valve Tissue Engineering





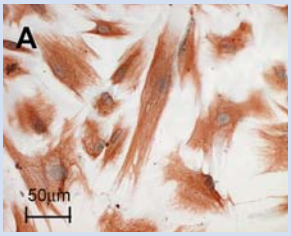


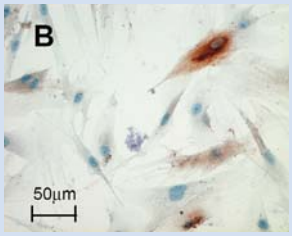


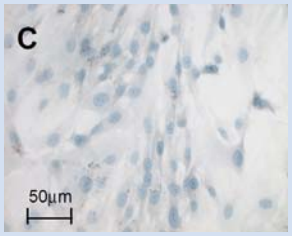
Heart valve tissue engineered from human marrow stromal cells

Hoerstrup et al. Circulation 2002

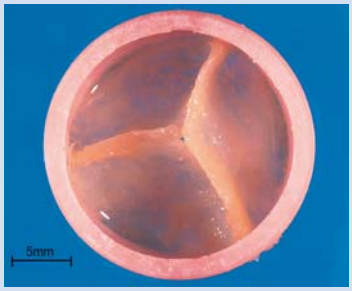
Human Prenatal Stem Cells for Pediatric Cardiovascular Tissue Engineering







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

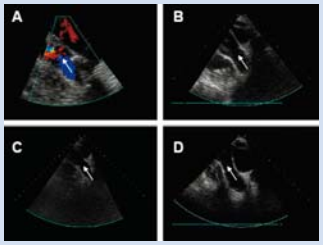


Heart valve tissue engineered from human prenatal stem cells.

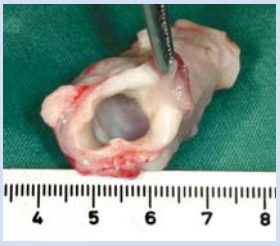
Schmidt D et al. Circulation 2006

Functional Growth in Living Cardiovascular Grafts

pre-implantation



in-vivo

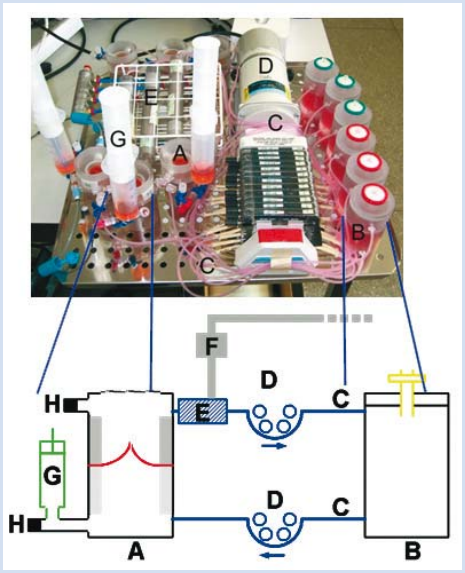


post-explantation

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

Hoerstrup SP et al. Circulation 2006

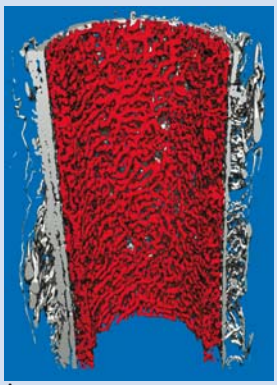
Bioreactor Development



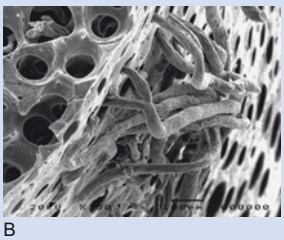
- sized small
- biocompatible materials
- maintenance of sterility
- static conditioning by continuous perfusion (4ml/min)
- dynamic conditioning by mimicking diastolic phase

Mol et al. Ann Biomed Eng 2005

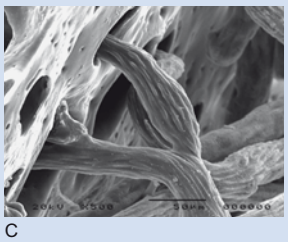
Neovascularisation of Biomaterials through Growth Factor Delivery



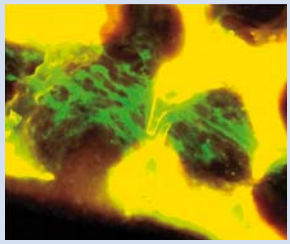
A



B



C

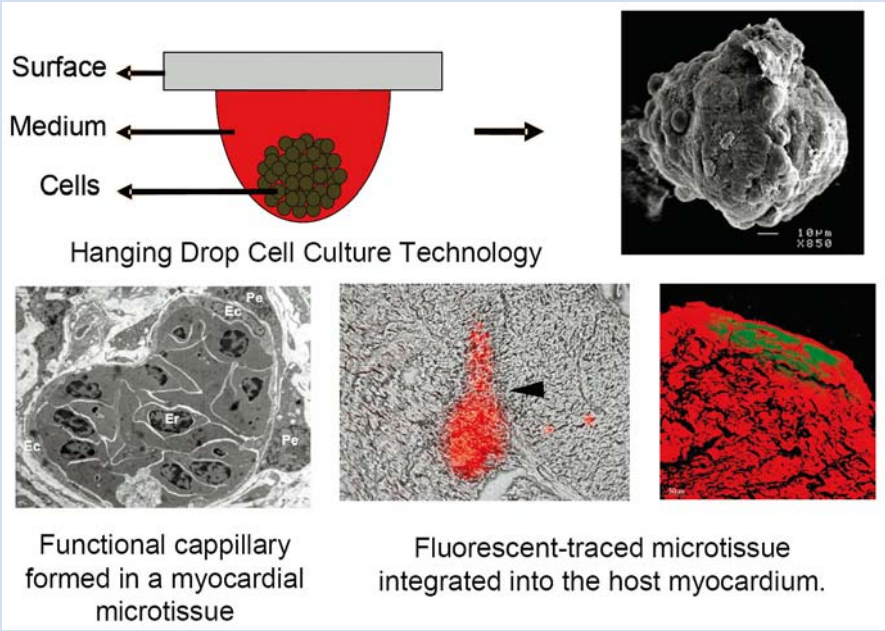


D

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

unpublished data

Design of Microtissues for Myocardial Regeneration



Myocardial microtissues are generated by gravity-enforced assembly of monodispersed cardiomyocytes in hanging drops. Myocardial microtissues (i) retain heart-muscle-specific morphology, (ii) express extracellular matrix components (iii) induce neovascularization, and (iv) integrate seamlessly into the myocardium after implantation into adult rats. Therefore they represent an attractive implantation format for cell-based therapy.
Kelm et al. Tissue Engineering 2006

Achievements 2006

- International Society for Applied Cardiovascular Biology, IACB-Young Investigator Award 2006, La Jolla, CA, USA, Schmidt D
- European Society of Artificial Organs (ESAO) Young Investigator Award 2006, Kelm J
- 5th Day of Clinical Research, Zurich, Switzerland: Best Poster Presentation 2006, Schmidt D
- Hartmann-Müller-Stiftung 2006: Schmidt D
- International Academic Scholarship Award 2006, Faculty of Health Sciences, University of Cape Town, South Africa, Schmidt C
- EU Grant Framework Program 6 (Biosys), Hoerstrup SP
- Bundesministerium für Bildung und Forschung (BMBF Grant), Hoerstrup SP
- Executive Committee, International Society of Applied Cardiovascular Biology, Akron, USA, Hoerstrup SP
- Expert Panel, Regenerative Medicine and Cellular Therapies, German Ministry of Education and Research (BMBF), Hoerstrup SP
- ZIHP Grant 2006 - 2008, Vulnerable atherosclerotic plaques - early detection, functional imaging, targeted treatment, Hoerstrup SP

Collaborations

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

Selected references:

- Schmidt D, Mol A, Odermatt B, Neuenschwander S, Breymann C, Gossi M, Genoni M, Zund G, Hoerstrup SP. Engineering of biologically active living heart valve leaflets using human umbilical cord-derived progenitor cells. Tissue Eng. 2006 Oct 1, Epub ahead of print
- Mol A, Rutten MC, Driessen NJ, Bouten CV, Zund G, Baaijens FP and Hoerstrup SP. Autologous Human Tissue-Engineered Heart Valves: Prospects for Systemic Application. (2006) Circulation 114 (I): I 152-158
- Schmidt D, Mol A, Breymann C, Achermann J, Odermatt B, Gössi M, Neuenschwander S, Prêtre R, Genoni M, Zund G and Hoerstrup SP. Living autologous heart valves engineered from human prenatally harvested progenitors. (2006) Circulation 114 (I): 125-131
- Hoerstrup SP, Cummings I, Lachat M, Schoen FJ, Jenni R, Leschka S, Neuenschwander S, Schmidt D, Mol A, Günter C, Gössi M, Genoni M, and Zund G. Functional Growth in Tissue-Engineered Living, Vascular Grafts: Follow-Up at 100 weeks in a large animal model. (2006) Circulation 114 (I): 159-166
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- Hofer CK, Ganter MT, Matter-Ensner S, Furrer L, Klaghofer R, Genoni M, Zollinger A. Volumetric assessment of left heart preload by thermodilution: comparing the PiCCO-VoLEF system with transoesophageal echocardiography. Anaesthesia (2006) 61 (4): 316-21
- Schmidt D, Asmis LM, Odermatt B, Kelm J, Breymann C, Gossi M, Genoni M, Zund G, Hoerstrup SP. Engineered living blood vessels: functional endothelia generated from human umbilical cord-derived progenitors. Ann Thorac Surg. 2006 Oct;82(4):1465-71
- Kelm JM, Djonov V, Hoerstrup SP, Guenter CI, Ittner LM, Greve F, Hierlemann A, Diaz Sanchez, Bustamante C, Perriard JC, Ehler E, and Fussenegger M. Tissue-Transplant Fusion and Vascularization of Myocardial Micro- and Macrotissues Implanted into Chicken Embryos and Rats. Tissue Eng. 2006 Sep;12(9):2541-53
- Schmidt D, Hoerstrup SP. Tissue engineered heart valves based on human cells. Swiss Med Wkly. 2006 Sep 30;136(39-40):618-23. Review
- Kelm JM, Djonov V, Ittner LM, Fluri D, Born W, Hoerstrup SP, Fussenegger M. Design of custom-shaped vascularized tissues using microtissue spheroids as minimal building units. Tissue Eng. 2006 Aug;12(8):2151-60.
- Breymann C, Schmidt D, Hoerstrup SP. Umbilical cord cells as a source of cardiovascular tissue engineering. Stem Cell Rev. 2006; 2(2):87-92.
- Kelm JM, Diaz Sanchez-Bustamante C, Ehler E, Hoerstrup SP, Djonov V, Ittner L, Fussenegger M. VEGF profiling and angiogenesis in human microtissues. J Biotechnol. 2005 Aug 4;118(2):213-29
- Mol A, Driessen NJ, Rutten MC, Hoerstrup SP, Bouten, CV, Baaijens FP. Tissue engineering of human heart valve leaflets: a novel bioreactor for a strain-based conditioning approach. Ann Biomed Eng. 2005 Dec;33(12):1778-88
- Schmidt D, Mol A, Neuschwander S, Breymann C, Gossi M, Zund G, Turina M, Hoerstrup SP. Living patches engineered from human umbical cord derived fibroblasts and endothelial progenitor cells. Eur J Cardiothorac Surg. 2005 May; 27(5):795-800.
- 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, Sodian R, Daebritz S, Wang J, Bacha E A, Martin D P, Moran A M, Guleserian K J, Sperling J S, Hatsuoka S, Kaushal S, Vacanti J P, Schoen F J, Mayer J E (2000) Functional living trileaflet heart valves grown in vitro. Circulation 102 (III): 44-49
- Kelm JM, Djonov V, Ittner LM, Fluri D, Born W, Hoerstrup SP, Fussenegger M Design of custom-shaped vascularized tissues using microtissue spheroids as minimal building units. Tissue Eng. 2006 12 (8):2151-60
- Maeder MT, Wolber T, Kunzli A, Genoni M, Blank R, Rickli H. Aortopulmonary fistula occurring 4 years after replacement of the ascending aorta. Ann Thorac Surg. 2006 81(5):e18-20

2.1.2 Ischemia / Reperfusion Injury

PD Dr. med.
Reza Tavakoli

Dr. sc. nat.
Inna Agarkova

Dr. sc. ETH
Roman Schönauer

Dr. rer. nat.
Anna Bogdanova

Dr. Deyan Mihov

Antiapoptotic effect of Erythropoietin in myocardial reperfusion injury

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

Aim of the project:
The present study aims to characterise the impact of apoptosis in ischemia-reperfusion injury and the efficiency of erythropoietin (Epo) known as a potent anti-apoptotic agent in cardioprotection.

Methods:
In our study we have used heterotopic heart transplantation model limiting the time of cold global ischemia of the graft to 45 min and varying the reperfusion time from 5 min to 24 h. Epo (5000 U/kg) was administered iv into the recipient animals 20 min prior to the onset of reperfusion of the graft.
To assess the myocardial damage we monitored plasma TnT levels and performed histological examination of the ventricular tissue of the graft.
In addition we measured plasma levels of cardiac stress hormones (ANP and BNP), tissue ion and water content and redox state markers (GSH:GSSH)
The following markers of apoptosis were chosen to evaluate the impact of apoptosis into the reperfusion-induced injury: caspase 3, caspase 9, PARP/ cleaved PARP and CardioTACS in situ apoptosis assay (RnD) to evaluate DNA fragmentation.

Results:
Pre-treatment of the recipient animals with Epo facilitated recovery of contractile function of the graft shortening the time before defibrillation from 74±7 sec in control vs 47±3 sec in Epo-treated animals. Whereas ANP levers in plasma were not reduced by Epo administration, plasma BNP content was significantly lowed in Epo-treated group (Figures 1 und 2).

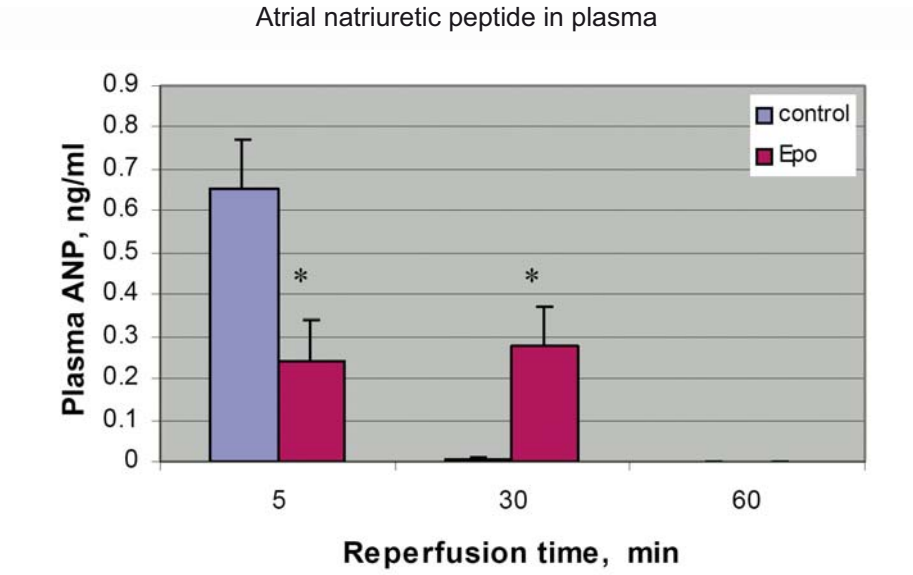

Figure 1

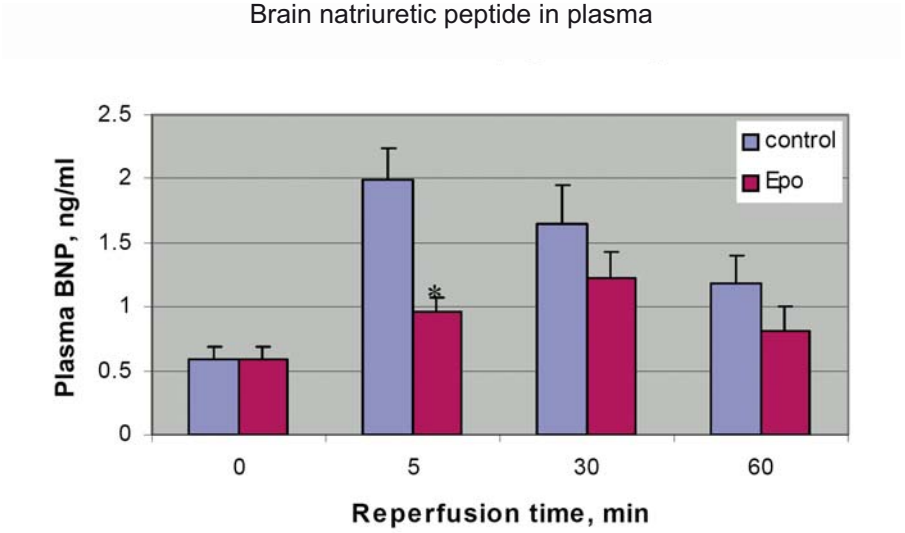

Figure 2

Epo treatment significantly decreased ischemia-reperfusion-induced myocardial damage as follows both from the levels of plasma TnT (Figure 3) and histological examination.

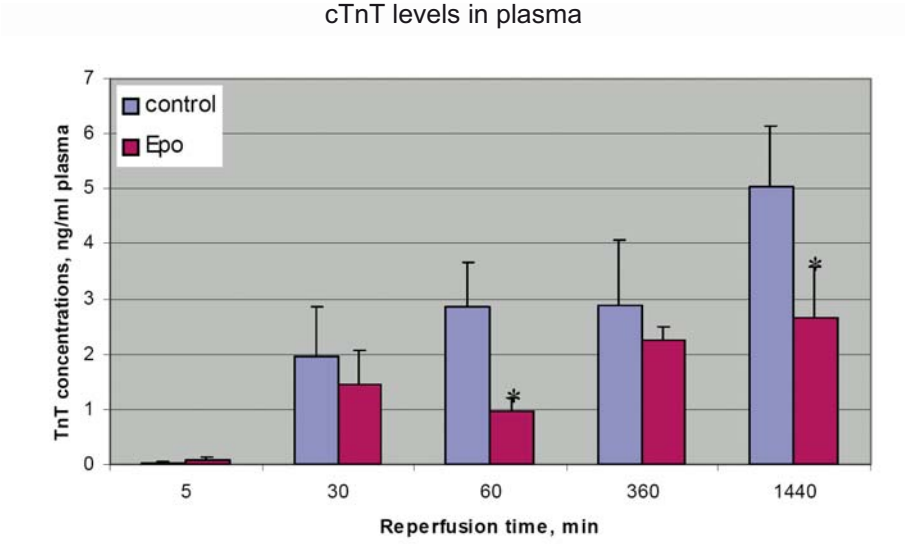

Figure 3

So far we were unable to detect any traces of apoptosis in the grafts of the untreated controls even though many of them showed extensive myocardial degeneration. Furthermore, microscopic examination as well as analysis of water/Na levels in the transplanted tissue revealed extensive swelling and Na+ accumulation in the damaged areas that it typical for necrosis. The obtained data allows us to suggest that apoptosis does not play a major role in tissue damage induced by ischemia-reperfusion. However, Epo was capable to successfully reduce myocardial damage acting not as an anti-apoptoic agent but reducing myocardial stress and necrosis.

Future plans:
We plan to elucidate possible targets of Epo action focusing on discrimination between the vascular bed as well as blood cells versus action of Epo at the level of myocardium. Furthermore, we shall dissect signal transduction pathways involved in cardioprotective effect of Epo with major emphasis on Act pathway and cytoskeletal integrity.

Achievements 2006

- Bogdanova A., Tavakoli R., Ossent P., Grenacher B., Bogdanov N., Zünd G., Genoni M., Gassmann M. Multi-parametric characterisation of ischemia-reperfusion injury: efficiency of antioxidant treatment
  Presented at Swiss cardiovascular research and training network, Bern, Jan. 27.
- Bogdanova A. Antioxidants in prevention of cold global ischemia-reperfusion injury of myocardium
  Invited lecture at the Seminar of the Institute of Veterinary Pathology University of Zurich May 29.
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  Presented at the 7th international Lübeck conference on the pathophysiology and pharmacology of erythropoietin and other hematopoietic growth factors, Lübeck, Germany, September 6-9.
- Mihov D., Grenacher B., Gassmann M., Bogdanova A., Tavakoli R. Erythropoietin and Li+ in protection of the heart against cold global ischemia-reperfusion injury
  Presented at the 2d ZHIP symposium, Zürich, September 22.
- Bogdanova A., Gerancher B., Ossent P., Gassmann M., Tavakoli R. Antioxidants in treatment of ischemia-reperfusion injury: mechanisms unknown?
  Presented at the annual meeting of the German Society of microcirculation and vascular biology, München, Germany, October 12-14.

Collaborations:

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

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2.1.3 Mechanical circulatory support

PD Dr. med.
Mario Lachat

PD Dr. med.
Markus Wilhelm

PD Dr. Mario Lachat, PD Dr. Markus Wilhelm

Long-term support

For left ventricular support, the Berlin Heart INCOR, a magnetically suspended and intracorporeally implanted axial-flow pump for left ventricular support, was used. Until end of 2006, eleven patients were supported with this device (fig. 1). Six patients were transplanted successfully, and one patient was still on support (454 days on 12/31/06). Six patients spent the waiting time for heart transplantation at home, and three patients went back to work while being on support.

The Berlin Heart EXCOR is an extracorporeally located pulsatile pump (fig. 2). It is used for biventricular or right ventricular support. Four patients were supported with the EXCOR. Three patients were discharged home with the device and wait for heart transplantation.

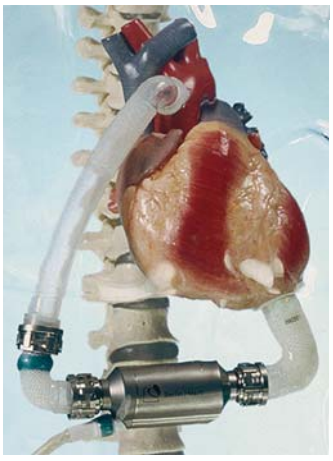


Figure 1 Berlin Heart INCOR

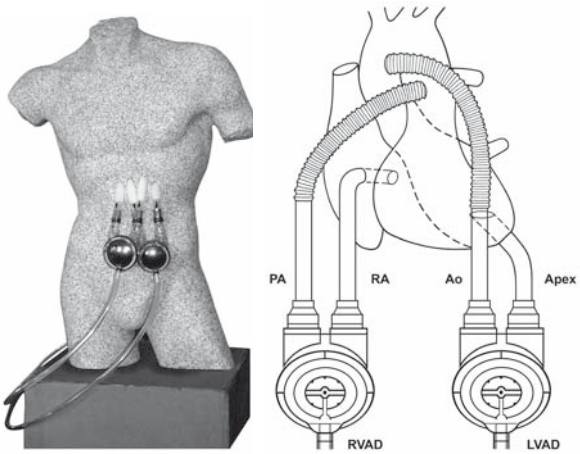
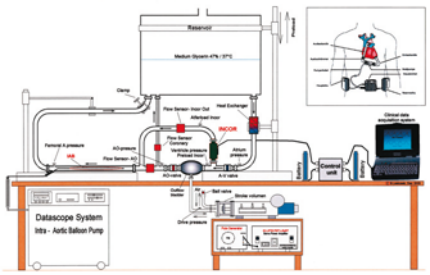


Figure 2 Berlin Heart EXCOR (links: extrakorporale Lage, rechts: Implantationsprinzip)

Short-term support

In 2006, 28 patients were supported with ECMO (extracorporeal membrane oxygenation) for acute heart and lung failure, respectively. In acute heart failure, veno-arterial ECMO was implanted in patients with postcardiotomy heart failure, and as rescue therapy in patients with rapidly developing cardiogenic shock as bridge to long-term mechanical support or transplantation. In lung failure, veno-venous ECMO was implanted in patients with ARDS due to causes such as fulminant pneumonia. ECMO support extended up to 4 weeks with good mechanical reliability.

INCOR (Berlin Heart) - Performance in vitro with IABP



Achievements 2006

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

Collaborations:

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

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2.1.4 Robotic Surgery and Innovative Technologies

PD Dr. med.
Jürg Grünenfelder

Dr. med.
André Plass

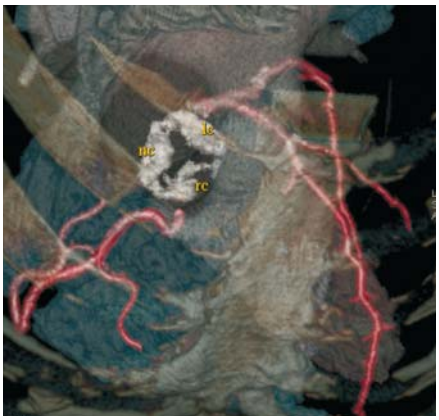
Dr. med.
Sacha Salzberg

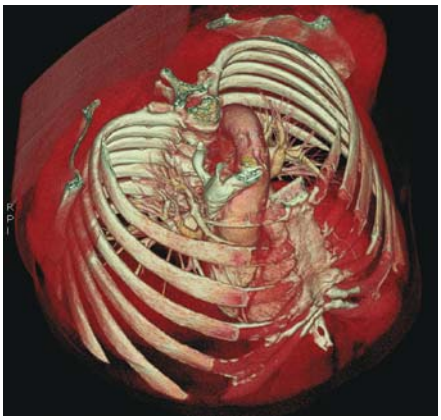
Minimally invasive cardiac surgery

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

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

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





Achievements 2006

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

Collaborations:

- Swiss national scientific foundation grant for computer aided and image guided medical intervention (CO-ME)
- Department of Radiology, Universtiy Hospital Zürich (Hatem Alkadhi, MD)
- Laboratory for Thermodynamics in Emerging Technologies, ETH Zürich (Prof. Dimos Poulikakos)
- Institute of Mechatronic Systems, ZHW (Prof. Van de Venn)
- Physical Electronics Laboratory, ETH Zürich (Prof. Henry Baltes)

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2.1.5 Congenital Heart Surgery

Prof Dr. med.
Rene Pretre

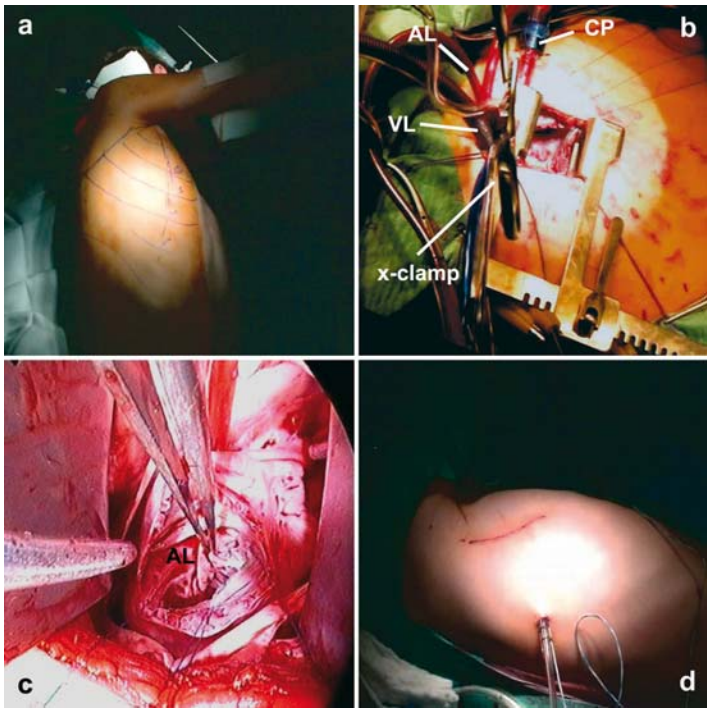
PD Dr. med.
Ali Dodge-Khatami

Prof. Dr. med. René Prêtre, PD Dr. med. Ali Dodge-Khatami

Projects

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

It is currently investigating on angiogenesis and pulmonary artery growth during single ventricle physiology, in a sheep model of cavo-pulmonary anastomosis. The surgeons are implicated in the validation and testing of a new equine jugular vein as a pulmonary valve substitute, and are designing a new model for transcutaneous-transdiaphragmatic insertion of a biological pulmonary vein prosthesis, under echocardiography guidance.



Achievements 2006

- Expansion of successful muscle-sparing mini-thoracotomy approaches to increasing diagnoses:

1. Repair of congenital heart defects (atrial septal defect, partial anomalous pulmonary venous return, ventricular septal defect, partial atrio-ventricular canal) using cardiopulmonary bypass through a right axillary mini-thoracotomy
2. Insertion of pacemaker electrodes and generator for left heart pacing through a muscle-sparing left axillary thoracotomy, constituting the world's largest published series of left heart pacing in children.

Collaborations:

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

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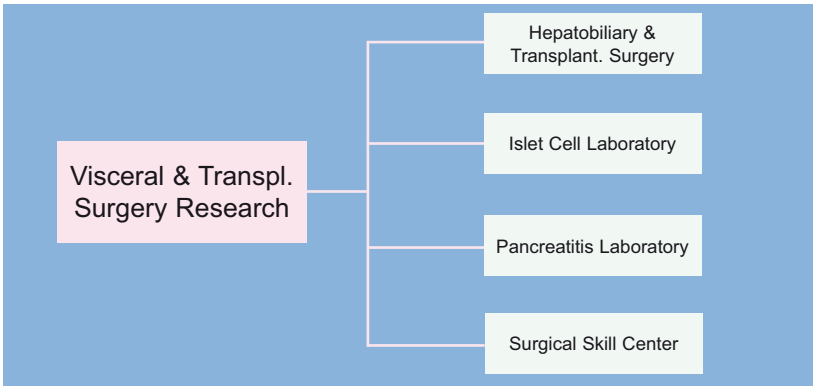
- Long-term follow-up after pacemaker implantation in neonates and infants. NC Aellig, C Balmer, A Dodge-Khatami, M Rahn, R Prêtre, U Bauersfeld. Ann Thorac Surg. 2006 Epub ahead of print.
- Initial experience with implantable cardioverter defibrillator systems using epicardial and pleural electrodes in paediatric patients. U Bauersfeld, M Tomaske, A Dodge-Khatami, M Rahn, CI Kellenberger, R Prêtre. Ann Thorac Surg. 2006 Epub ahead of print

2.2 Visceral & Transplant Surgery Research

PD Dr. phil II
Rolf Graf

Prof. Dr. med.
Pierre-Alain Clavien

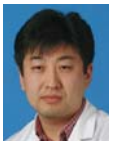


2.2.1 Hepatobiliary & Transplantation Surgery

PD Dr. med.
Yinghua Tian

Dr. med.
Panco Georgiev

Dr. med.
Ashraf Osman



Dr. nat.
Jae-Hwi Jang PhD

Dr. med.
Antonio Nocito

Udo Ungethüm

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

Ischemia/reperfusion injury of the liver represents an important problem in major hepatic surgery and liver transplantation. Two types of injury have to be differentiated: (1) warm ischemic injury, occurring during liver resection, (2) cold ischemic injury, occurring during organ preservation. Our group has a longstanding interest in the cellular processes leading to these two types of injuries. Additional factors affecting the outcome after ischemia and reperfusion are the presence of steatosis, cholestasis, cirrhosis and the age of the liver.

To assess ischemic injury in the fatty liver, intravital microscopy was used. We could show that the type of steatosis (macro- versus microvesicular steatosis) has a strong impact on various parameters of perfusion and activity of leukocytes. Protective strategies e.g. ischemic preconditioning and intermittent clamping could improve these parameters. These results are in accordance with a recent publication in which the impact of steatosis on liver injury after ischemia and reperfusion was demonstrated *(Selzner et al., J.Hepatology 2006)*.

Currently, investigations into the ability of the old liver to tolerate ischemic injury are performed. Results indicate that old livers are more prone to tissue injury which can be partially corrected by supplying glucose prior to a surgical insult. Here we could show that the ATP content of the liver was dramatically increased after a supplement of glucose. Concurrently, the injury was strongly reduced.

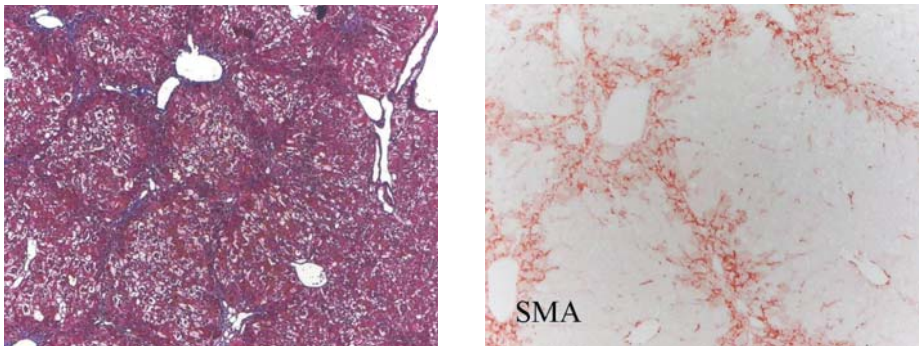
The mouse orthotopic liver transplantation (OLT) model was further developed: it was shown that 30% transplantation of a mouse liver results in a small for-size syndrome which results in morbidity and mortality. Various approaches

to protect the mouse from a small-for-size liver syndrome resulted in the detection of a mechanism involving TNF-α and Kupffer cells. Indeed, application of pentoxifylline, a drug routinely used in clinical therapy for thrombosis, was successful in reverting the small-for-size syndrome. Pentoxifylline suppresses TNF-α synthesis and has rheological activities, improving blood circulation *(Tian et al. 2006, PNAS, 103:4598-603)*.

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

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

An additional project is currently focusing on the cirrhotic liver. We are trying to understand whether surgical and pharmacological strategies can protect the cirrhotic liver from ischemic injury. A model of liver cirrhosis was used that exhibits extensive tissue rearrangement (CCl_4).



Histology of cirrhotic mouse liver. H&E staining (left) and immunostaining for α-SMA, indicating activated stellate cells (right).

The role of serotonin in ischemia and reperfusion injury

Hepatic ischemia and reperfusion (I/Rp) leads to the formation of leukocyte-platelet aggregates. Upon activation, platelets generate reactive oxygen species and release proapoptotic and proinflammatory mediators as well as growth factors. In cold hepatic ischemia adhesion of platelets to endothelial cells mediates sinusoidal endothelial cell apoptosis. Furthermore, platelet-derived serotonin mediates liver regeneration. We therefore hypothesized that platelets might be mediators of reperfusion injury after normothermic hepatic ischemia.
To understand the impact of platelets in normothermic hepatic I/Rp injury we used models of impaired platelet function and immune thrombocytopenia. Inhibition of platelet function in mice was achieved by clopidogrel feeding. Immune thrombocytopenia was induced by intraperitoneal injection of anti-CD41 antibody. Serotonin deficiency was studied in a mouse model lacking tryptophan hydroxylase-1 (*Tph1*), a key enzyme for the biosynthesis of peripherial serotonin. All mice were subjected to 60 minutes of partial hepatic ischemia and various time points of reperfusion.
Neither inhibition of platelet function nor platelet depletion led to a reduction of I/Rp-injury. In contrast, liver regeneration and repair were significantly impaired in platelet depleted animals. Mice lacking peripheral serotonin were deficient in hepatocyte proliferation, but displayed normal repair.
Therefore, we conclude that platelets have no direct impact on the pathogenesis of normothermic I/Rp injury. However they mediate tissue repair and liver regeneration. Furthermore, platelet-derived serotonin is a mediator of hepatocyte proliferation in the postischemic liver, but has no impact on tissue remodeling and repair.

Prevention of reperfusion injury and microcirculatory failure in macrovesicular steatotic mouse liver by omega-3 fatty acids

We have previously shown that macrovesicular hepatic steatosis has a lower tolerance to reperfusion injury than microvesicular steatosis. To further investigate the mechanism of injury, we analyzed the fat contents of obese livers. Obese mice with macrosteatotic livers exhibited an abnormally high ratio of omega-6:omega -3 polyunsaturated fatty acids (PUFAs), more so than livers with microsteatosis. Therefore, we investigated the influence of PUFAs on microcirculation in steatotic livers and the potential to minimize reperfusion injury in the macrosteatotic liver by normalization of PUFAs. *Ob/ob* mice were used as a model of macrovesicular hepatic steatosis and C57/Bl6 mice fed a choline deficient diet for microvesicular steatosis. Steatotic and lean livers were subjected to 45 minutes of ischemia and 3 hours of reperfusion. Microcirculation was investigated using intravital fluorescence microscopy. A second group of *ob/ob* mice was supplemented with dietary omega-3 PUFAs and compared to control diet fed group. Macrosteatotic livers had significant microcirculatory dysfunction correlating with high omega-6:omega-3 PUFA ratio. Dietary omega-3 PUFA supplementation resulted in normalization of this ratio, reduction of intrahepatic lipids and decrease in the extent of macrosteatosis. Defective microcirculation was dramatically ameliorated with significant protection against hepatocellular injury both before ischemia and after reperfusion.

We conclude that macrosteatotic livers disclose abnormal omega-6: omega-3 PUFA ratio. This correlates with microcirculatory defect and enhanced reperfusion injury. Thus, protective strategies applied during or after ischemia are unlikely to be useful. However, preoperative dietary omega-3 PUFAs protect macrosteatotic livers against reperfusion injury and might represent a valuable method to expand the live liver donor pool.

Achievements 2006

Scientific

- We used a unique model of arterialized partial liver transplantation in mice. We could show that recipients of small-for size livers (30%) have an improved survival rate after treatment with pentoxifylline.
- Establishment of mouse models of cholestasis
- The role of energy charge in aging livers: pre-treatment of animals with glucose reduces ischemic injury of the liver.
- Platelets have no direct impact on the pathogenesis of normothermic I/R injury, but mediate tissue repair and liver regeneration.
- Investigations into the mechanism of ischemia reperfusion injury in fatty livers led to the conclusion that these livers exhibited reduced sinusoidal perfusion and impaired leukocyte function.
- Preoperative dietary omega-3 PUFAs protect against reperfusion injury in a mouse model of fatty liver.

Personnel

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

Collaborations:

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

Selected references:

- Dahm F, Georgiev P, Clavien PA. Small-for-Size Syndrome After Partial Liver Transplantation: Definition, Mechanisms of Disease and Clinical Implications. Am J Transpl 2005; 5:2605-2610.
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- Y. Tian, W. Jochum, P. Georgiev, W. Moritz, Rolf Graf, and P.A. Clavien. Kupffer Cell dependent TNF-α Signaling mediates injury in the Arterialized Small-for-Size Liver Transplantation in the Mouse. PNAS 2006, 103:4598-603
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  Bile salt toxicity aggravates cold ischemic injury of bile ducts after liver transplantation in Mdr2+/- mice. Hepatology 2006;1022-31.

Dr. med.
Katarzyna Furrer

Dr. med.
Christopher Soll

Dr. med.
Mickael Lesurtel

Liver regeneration

Dr. med. K. Furrer, Dr. med. Christopher Soll, Dr. med. Mickael Lesurtel

The liver is the only solid organ with the capacity to regenerate its volume after major tissue loss. Previous studies have shown that platelets are involved in a critical step during regeneration. To identify mediators of hepatocyte proliferation, soluble factors generated by platelets were investigated. One possibility was serotonin which is synthesized by the intestine and taken up by platelets. We could show that serotonin is a key mediator of liver regeneration. A two-step enzymatic reaction converts tryptophane to serotonin. The first step depends on tryptophane hydroxylase-1(TPH-1) which converts tryptophane into 5-hydroxytryptophane (5-HTP). When TPH-1 knockout mice were used in experiments in which 70% of the liver was removed, regeneration was strongly reduced *(Lesurtel et al. Science 2006)*. Regeneration could be rescued by supplementing these mice with 5-HTP. To further identify molecular interactions between hepatic cells, we have started to isolate hepatocytes, sinusoidal epithelial cells and Kupffer cells. We have been able to successfully culture these cells with a high viability and purity, as determined by FACS analysis. Individual cell types will be exposed to various mitogenic factors to test proliferation.

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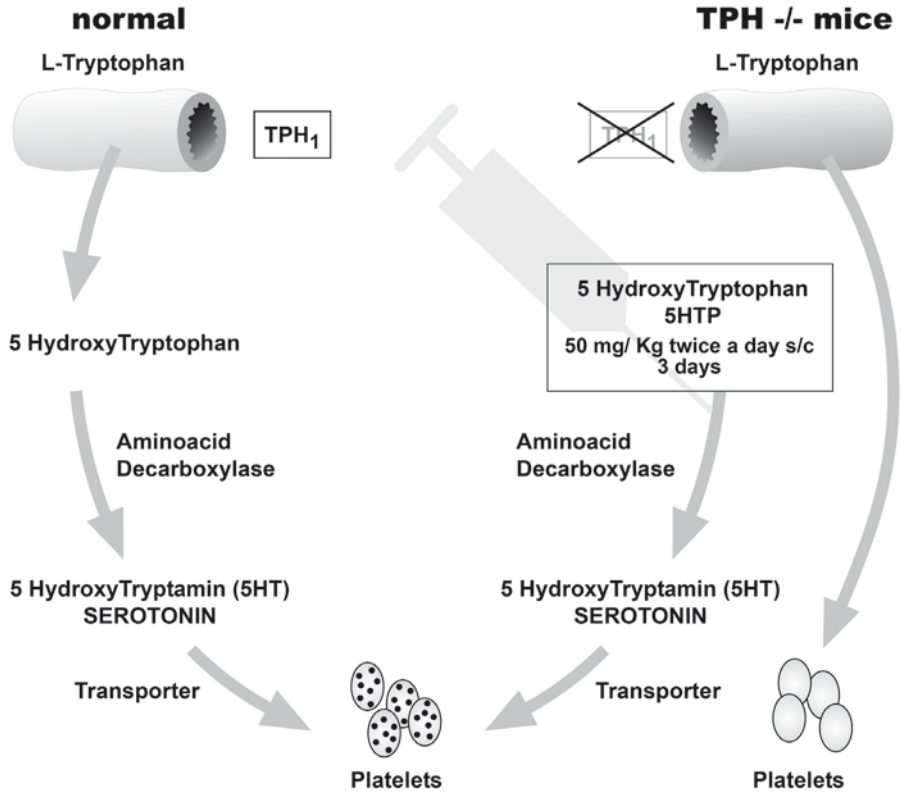
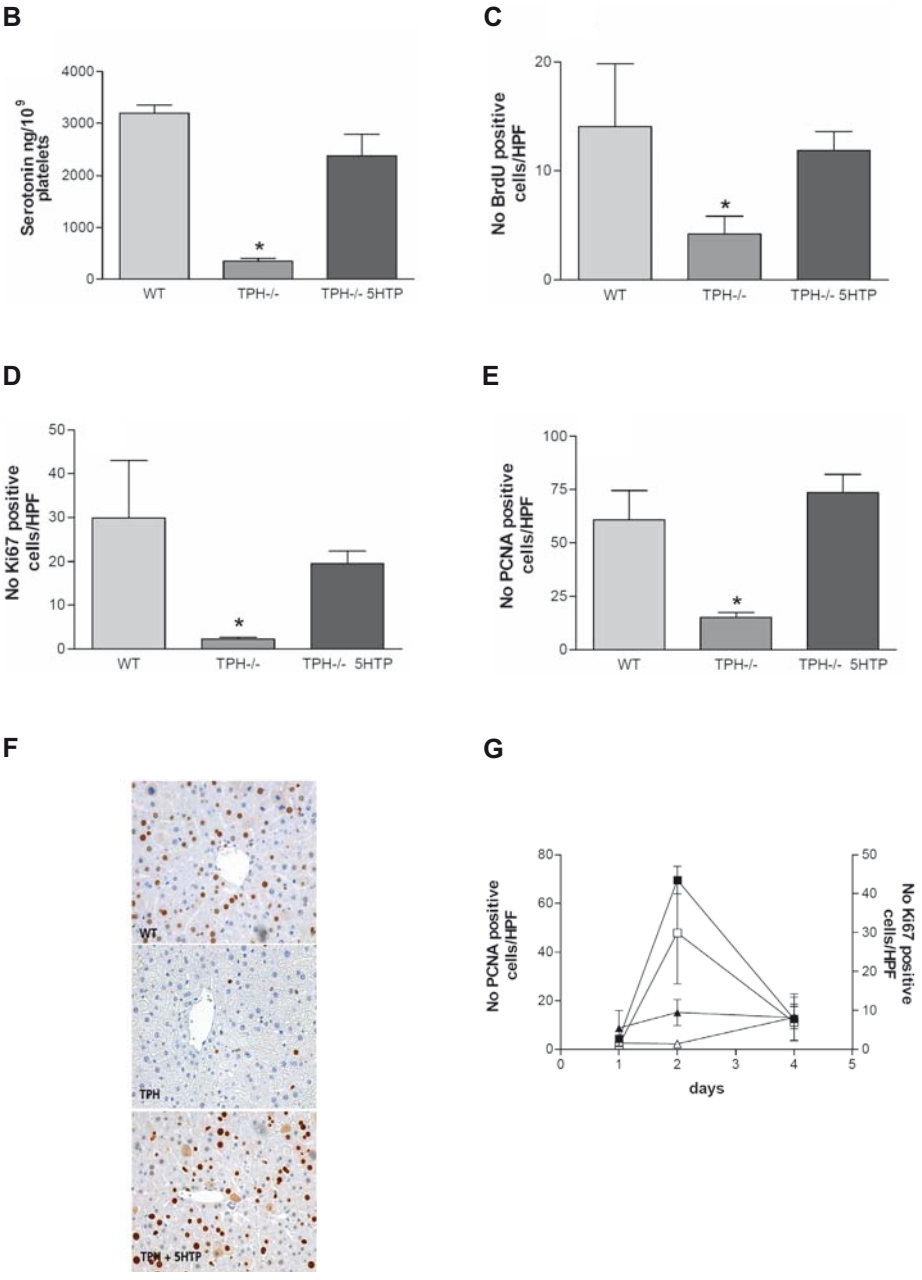




Effect of serotonin on liver regeneration. A: Experimental scheme: in wild type animals, tryptophan in the small bowel is converted to 5-hydroxytryptophan (5-HTP) by tryptophan-hydroxylase 1 (TPH1) which is then further converted by the ubiquitous amino acid decarboxylase to serotonin. Serotonin is taken up by platelets through the serotonin transporter. In the TPH1 knock-out mouse (TPH1-/-), tryptophan is not converted to 5-HTP and hence the platelets lack serotonin. In reloading experiments in TPH1-/- mice, whereby 5-HTP is supplemented, the conversion to serotonin proceeds normally. **B**: Serotonin levels in thrombocytes of wild type (WT) mice, TPH1-/- mice and TPH1-/- mice after supplementing with 5-HTP (TPH1-/- 5-HTP). **C**: Effect of serotonin depletion in platelets on hepatocyte proliferation two days after hepatectomy. The number of BrdU positive cells in wild type (WT), TPH1-/- and TPH1-/- mice supplemented with 5-HTP (TPH1-/- 5-HTP). **D, E** number of Ki67 and PCNA positive cells in the same liver remnants. **F**: histological examples of PCNA stained sections from remnant livers. **G**: Time course of labelling indexes for PCNA (■,▲) and Ki67 (□,Δ) in hepatectomized wildtype (■,□) and TPH-/- livers (▲,Δ).

Regeneration in embolized and ligated livers

Liver resection is the only cure for a number of malignant or benign diseases. Preoperative induction of hypertrophy of the healthy part of the liver increases the resectability rates of large tumors. Two strategies are clinically available to induce selective hypertrophy of the liver; portal vein emobilzation (PVE) and portal vein ligation (PVL). To investigate the rate of regeneration after these treatments, male Wistar rats were subjected to selective 70% PVL, 70% PVE, as well as 70% partial hepatectomy (PH) or sham operation. PVE of liver segments was validated by angiography and histological staining demonstrating permanent obstruction of the involved portal branches.
The weight of the regenerating liver segments increased in all groups reaching the highest values after PH. This group also disclosed the strongest proliferative activity. For example, Ki67 staining at 48h disclosed 280 positive cells per visual field (VF) vs. 10 in the sham group. PVL displayed also a high regenerative response, which was significantly more pronounced than PVE ($p=0.01$). The ipsilateral part of the liver atrophied in both groups with smaller volumes observed in the PVL group. We conclude that selective induction of liver hypertrophy is highly dependent on the type of intervention, with PH associated with the strongest regenerative response. Surprisingly, PVL was superior to PVE both in the regenerative response of the contralateral segment and atrophy of the ipsilateral part of the liver. These findings may trigger the search for novel strategy in patients.

Achievements 2006

Scientific

- Demonstration that serotonin is a key mediator in liver regeneration
- Serotonin is not involved in ischemic injury but promotes regeneration

Personnel

- Dr. M. Lesurtel received the SGC award for his lecture at the annual meeting of the Swiss Society of Surgery.

Collaborations:

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

Selected references:

- Lesurtel M, Graf R, Aleil B, Walther DJ, Tian Y, Jochum W, Gachet C, Bader M, Clavien PA, Platelet-derived serotonin mediates liver regeneration. 2006, Science 12:104-7

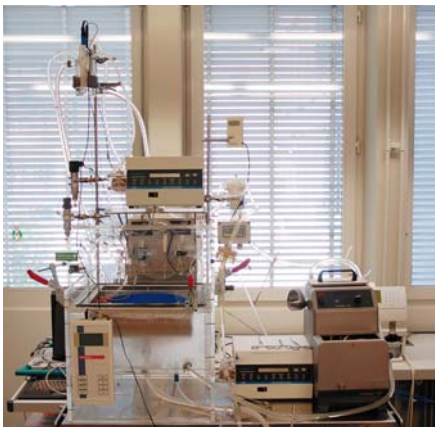
PD Dr. med.
Philipp Dutkowski

Hypothermic oxygenated pig liver perfusion

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

During cold storage of livers cellular components deteriorate resulting in energy depletion and loss of function. Tissue damage from cold storage is aggravated by rewarming and reperfusion. The combined tissue injury eventually leads to a loss of the graft. Previously, we have demonstrated that hypothermic oxygenated perfusion (HOPE) improved several parameters including lipid peroxidation, bile flow, energy charge, total glutathione and LDH release in rat livers after cold storage. *(Dutkowski et al., American J. Transplantation 2006)*

In a further attempt to reach a clinically acceptable system with which livers can be 'recharged' after ischemia we designed and constructed a perfusion system in which pig livers can be maintained. Pig livers were obtained from the slaughterhouse and perfused for several hours. Pig livers after extended warm ischemia and cold storage exhibited considerable loss of function while livers without warm ischemia were much better preserved. We now aim to 'recharge' livers after warm ischemia and cold storage using HOPE in the machine perfusion system. This proof of principle will be a key step to evaluate whether HOPE in a machine perfusion system is feasible in a clinical setting.



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

Achievements 2006

Scientific

- Establishement of an isolated perfused pig liver system

Selected references:

- Dutkowski P., Graf R, Clavien PA. Rescue of the cold preserved liver by hypothermic oxygenated machine perfusion. 2006 American J. Transpl. 6:903-12
- Dutkowski P, Furrer K, Tian Y, Graf R, Clavien PA. Novel short-term hypothermic oxygenated perfusion (HOPE) system prevents injury in rat liver graft from non-heart beating donor. Ann Surg. 2006;244:968-76

Dr. med.
Stefan Heinrich

Dr. med.
Felix Dahm

Dr. med.
Daniel Dindo

Dr. med.
Michelle de Oliveira

Oncology

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

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

Another project focuses on the role of sphingolipids in cancer cell death and survival. Sphingolipids are a complex class of lipids that not only constitute cellular membranes, but also play an important role in intra- and extracellular signalling. The sphingolipid ceramide plays an important role in the induction of apoptosis. By using liquid chromatography and mass spectrometry we could show that specifically targeted cationic ceramide analogues accumulate in mitochondria in vitro. These compounds exert cytotoxic effects on a variety of cancer cell lines. We studied the human colorectal cancer cell line SW403 in detail and showed a decreased mitochondrial membrane potential, cytochrome c release and cytoplasmic caspase activation. Cells reacted to the ceramide compound by an overall decrease of endogenous ceramide, and a rapid rise of sphingosine-1-phosphate, a sphingolipid counterplayer of ceramide. In vivo we could establish a tolerable dosing regime along with pharmacokinetic behaviour of the ceramide compound. Most importantly, ceramide treatment reduced the growth of subcutaneous tumour metastases in vivo. Targeting mitochondria with ceramide analogues offers a promising new approach to cancer treatment.*(Dindo et al., Mol. Cancer Therapy 2006)*

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

Achievements 2006

Scientific

- Colorectal cancer cells reduce liver regeneration by producing TGFβ
- Cellular distribution of sphingolipids in blood

Collaborations:

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

Selected references:

- Dindo D, Dahm F, Szulc Z, Bielawska A, Obeid LM, Hannun YA, Graf R, Clavien PA. Cationic long-chain ceramide LCL-30 induces cell death by mitochondrial targeting in SW403 cells. Mol Cancer Ther. 2006 5:1520-9.
- Dahm F, Nocito A, Bielawska A, Lang KS, Georgiev P, Asmis LM, Bielawski J, Madon J, Hannun YA, Clavien PA. Distribution and dynamic changes of sphingolipids in blood in response to platelet activation. J Thromb Haemost. 2006, 4:2704-9.
- Heinrich St, Jochum W, Graf R, Clavien PA., Portal vein ligation and partial hepatectomy differentially influence growth of intrahepatic metastasis and liver regeneration in mice. J. Hepatology 2006 45:35-42

2.2.2 Islet Cell Laboratory

Dr. phil. II
Wolfgang Moritz

PD Dr. med.
Markus Weber

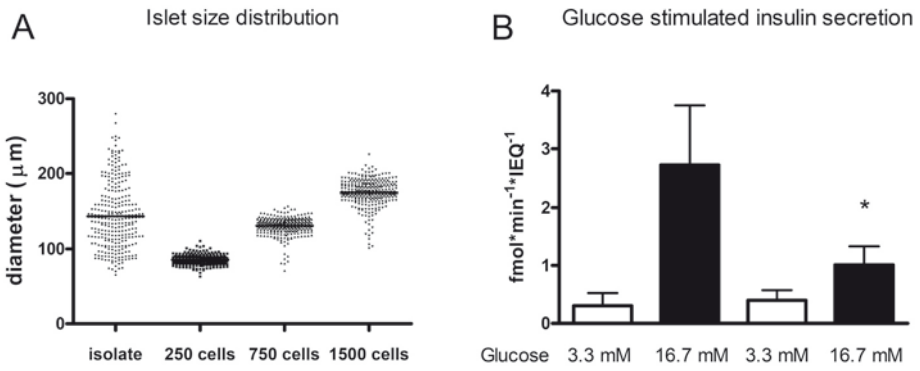
med. pract.
Patrick Kugelmeier

Lu Tuyet Trinh

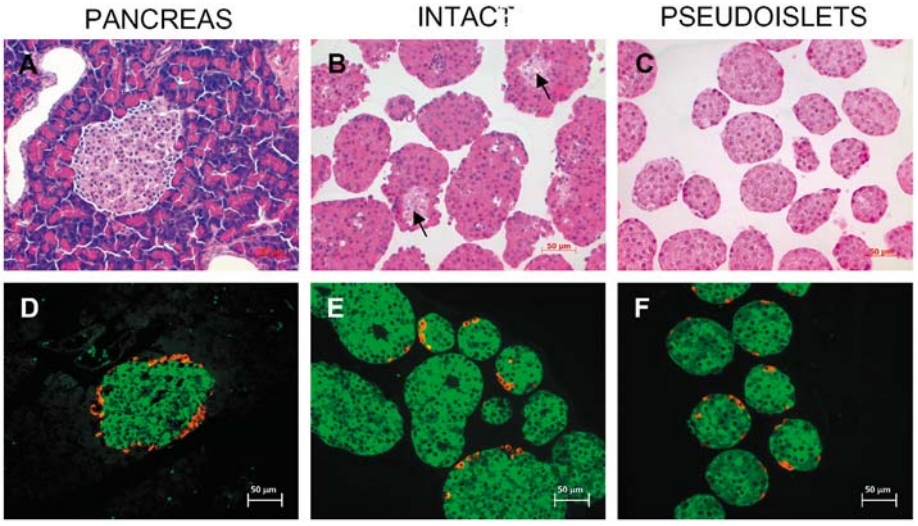
Tissue Engineering in Therapeutic Islets Transplantation
Dr. Wolfgang Moritz, PD Dr. med. Markus Weber

Our longstanding research interest focuses on strategies that improve the outcome and efficiency of islets transplantation, a therapeutic option for the treatment of type 1 diabetes mellitus. Such efforts are undertaken in close collaboration with the Unit of Endocrinololology and Diabeteolgy at the University Hospital of Zürich. Islets transplantation has become a widely accepted therapy in patients requiring a kidney transplant and where thus the administration of immunosuppressive drugs is unavoidable. Islets are isolated from pancreata of heart-beating donors and transplanted into the liver of diabetic recipients by transhepatic portal catherization. However, despite initial promising results, recent clinical follow-up studies indicate that islet graft function eventually declines over the years, with only approximately one forth of patients to be insulin indepent at 2 years after transplantation. Gradual graft loss may not solely be the result of organ rejection, but possibly also due to chronic overstimulation and metabolic exhaustion, particularly in situations of a marginal graft mass.

According to our own observations from a rat islet transplantation model, we found strong evidence of metabolic exhaustion based on insulin immunohistochemistry in transplanted islets. In diabetic rats, transplanted islets showed a marked reduction in insulin staining intensity, which seemed to be even more pronounced in large islets. This suggests that larger islets are prone to metabolic exhaustion, which is concordant to our observation that neovascularization of intrahepatically transplanted islets occurs predominantly at the islet periphery and only to a lesser extend within the islet core regions. Animal studies and observations from clinical islets transplantations suggest that islet transplants composed of mainly small islets exhibit improved graft function as compared to transplants consisting of mainly large islets. Our aim was therefore to generate small islets which are less susceptible to the lack of a functional vasculature and that can be supplied with nutrients and oxygen solely by diffusion. We therefore applied the so called “hanging drop”-technology with suspensions of dissociated rat islets cells. With this method so called pseudoislets of a defined and standardized size could be generated, which showed a similar cell composition and architecture like native islets from the pancreas. Necrotic cores as observed under standard culture conditions in large native islets were absent in small pseudoislets. Glucose stimulated insulin secretion in pseudoislets correlated with their intracellular insulin content and was increased by 2.5 fold when compared to size-matched native islets. Both, insulin content and insulin secretion was inversely correlated to islet size, which emphasizes the advantage of small islets in the absence of vascular perfusion. In conclusion, we demonstrate a feasible method to generate islets of a defined small size, which exhibit an identical morphological appearance as native islets but possess a dramatically enhanced functional capacity. Such knowledge is important not only to clinical islets transplantation, but also for future attempts to obtain and transplant islet cell replacement tissue by applying stem cell technology.



(A) Comparison of islet size from a regular isolation and from hanging drop cultures with cell suspensions of various cell densities. (B) Insulin secretory capacity of pseudoislets composed out of 250 cells compared to native islets, both normalized to islet size.



Histological comparison of native pancreatic islets (A, D), after isolation (B, E) and pseudoislets after reaggregation from a single cell supension (C, F). Paraffin sections were stained with H&E (A-C) or with antibodies directed against insulin (green) and glucagon (red) (D-F). Necrotic cores in intact islets are marked by arrows.

Achievements 2006

- We were able to demonstrate the feasibility of generating islets of defined dimensions with decreased susceptibility to ischemic injury.
- In vitro generated pseudoislets show an increased glucose stimulated insulin secretion.
- Financial support was obtained from the Olga-Mayenfisch 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 Dr. Kelm from the Tissue Engineering Group of Cardiovascular Research led by Prof. Hoerstrup.

Selected references:

- Moritz W., Meier F., Stroka D. M., Giuliani M., Kugelmeier P., Nett P.C., Lehmann R., Candinas D., Gassmann M., Weber M.
  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 2005:14: 67-76

2.2.3 Pancreatitis Research Laboratory

PD Dr. phil. II
Rolf Graf

PD Dr. med.
Daniel Bimmler

Dr. med.
Li K. Sun

Dipl. phil. II
Theresia
Reding Graf

Martha Bain

cand.med.
Federico
Storni

MCP-1 regulation in pancreatic acinar cells
Dr. med. Li-Kang Sun, Martha Bain, Dipl. phil. II Theresia Reding Graf, PD Dr. med. D. Bimmler

In human chronic pancreatitis inflammatory cells e.g. neutrophilic granulocytes and macrophages infiltrate the parenchyma. Inflammation is associated with a high increase of cyclooxygenase-2 (COX-2) activity. In a spontaneous model of pancreatic inflammation and fibrosis (WBN/Kob rat) we observed that inhibition of COX-2 led to a significant decrease of prostaglandin E_2 and proinflammatory cytokines and chemokines e.g. TNF-α, IL-6 MCP-1 and TGF-β. Furthermore, we could show that COX-2 was predominantly localized in macrophages
To test whether prostaglandin E_2 (PGE_2) is relevant in the response of the pancreatic acinar cells to stimuli by cytokines, we used two approaches: in vivo determination of PGE_2 receptors and in vitro stimulation in cell cultures.

In the first approach we determined the presence of PGE_2 receptors in the pancreas of Wistar rats and WBN/Kob rats. All receptors were present on the mRNA level in the pancreas of Wistar rats. In the WBN/Kob rats, there was a strong increase in PGE_2 receptor isoforms suggesting that they are involved in the process of chronic inflammation. In WBN/Kob rats that received a COX-2 inhibitor, PGE_2 receptors were much reduced, similar to or even lower than in Wistar rats.

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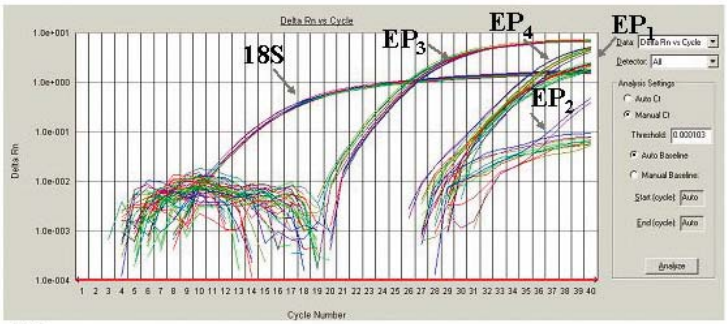
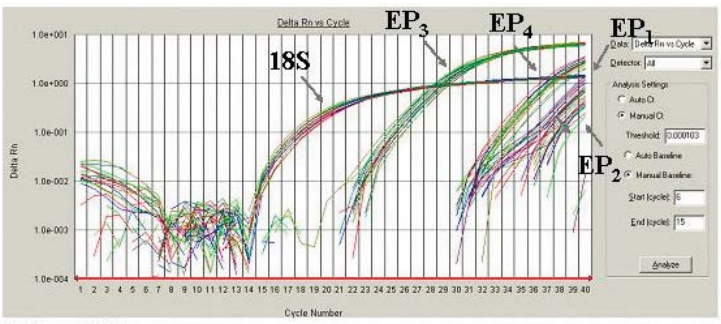
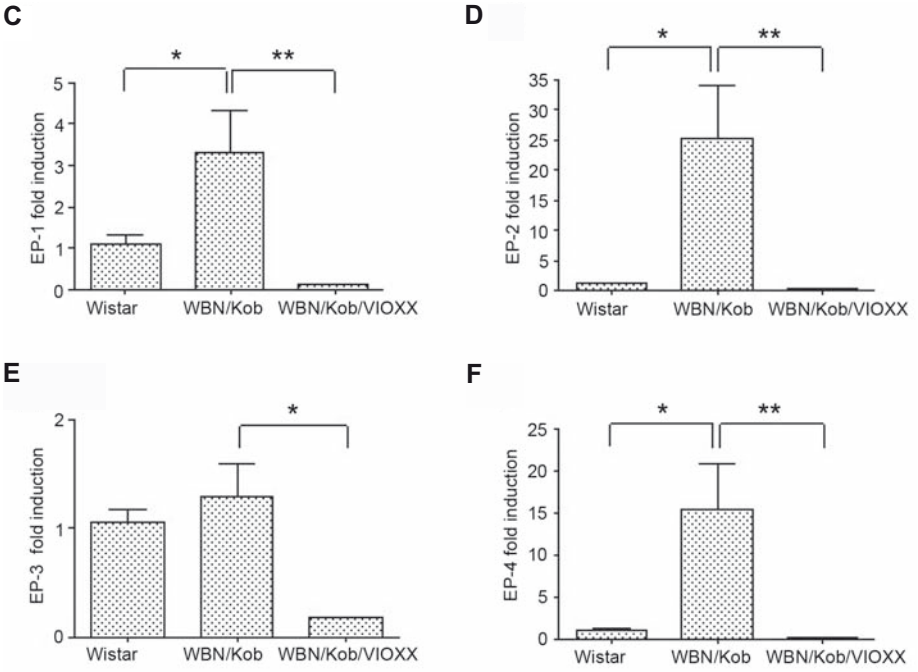


EP_{1-4}, AR42J cell line

B



EP_{1-4}, Wistar Healthy

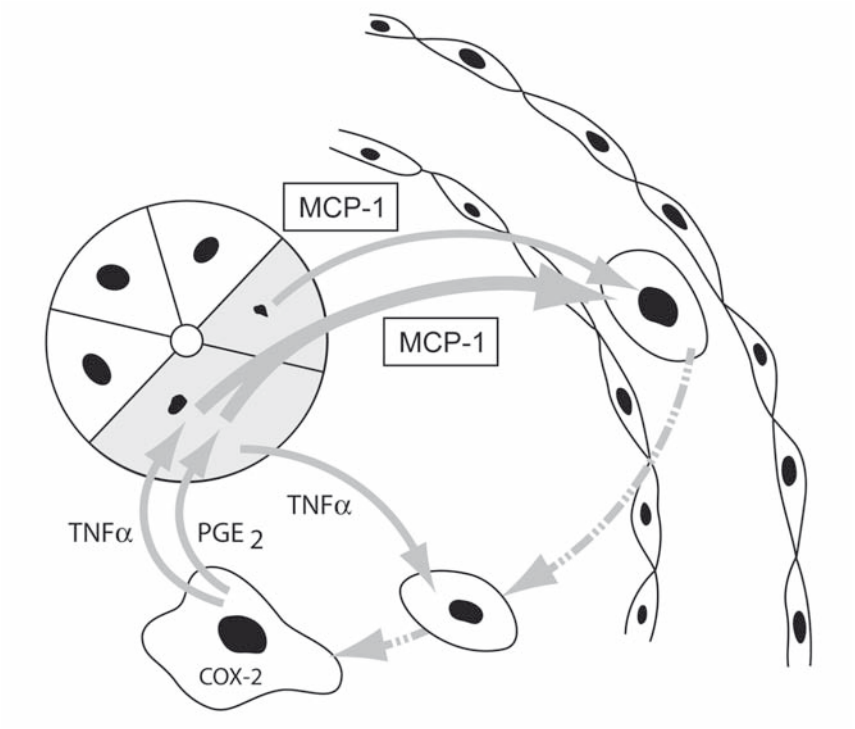


Presence of PGE_2 receptors EP1- EP4 in a pancreatic acinar cancer cell line AR42J (A) and in Wistar rat pancreas (B) demonstrated as amplification plots after reverse transcription and real time PCR. C-F demonstrate the regulation of the prostaglandin receptor isoforms in Wistar rats and WBN/Kob rats with and without treatment with a COX-2 inhibitor.

MCP-1 is a chemokine and plays and important role in recruitment of macrophages. In the WBN/Kob rat pancreas it is predominantly expressed in acinar cells. To test the effects of prostaglandins on pancreatic acinar cells, a pancreatic acinar cancer cell line was exposed to PGE_2 and TNFα to test whether these factors stimulated a response. We could show that both TNFα and the chemokine MCP-1 were stimulated. In combination TNFα and PGE_2 induced a synergistic mRNA synthesis of cytokines and chemokines.

In the next step we tested whether these observations were true in a primary cell culture system using pancreatic lobuli. Again, there was a synergistic secretion of MCP-1 after stimulation by both PGE_2 and TNFα.

To further explore the regulation of MCP-1, inhibitors of intracellular signalling cascades were applied. The synergistic action of TNFα and PGE_2 could be abolished by application of an protein kinase A inhibitor while a protein kinase C inhibitor was ineffective. To further substantiate this observation, western blots for CREB (cyclic AMP responsive element binding protein) were performed. This protein is phosphorylated by PKA. Indeed, CREB was phosphorylated when TNFα and PGE_2 were added to pancreatic lobuli. Application of a PKA inhibitor could inhibit this phosphorylation, suggesting that the synergistic effect of these factors is PKA dependent.
We conclude that the chemical interactions of macrophages and acinar cells might be a key step in establishing the chronic phase of pancreatitis.



Acinar cell damage leads to the secretion of MCP-1. Monocytes in blood vessels are are attracted to the site of injury and mature to macrophages. TNFα secreted by acinar cells activate or maintain activation of macrophages. These cells also produce and secrete various cytokines, chemokines, prostaglandins and TNFα. The combination of cytokines and TNFα then synergistically activates further acinar cells and propagate inflammation.

PAP does not protect the pancreas in CF-mice

Pancreatitis-associated protein (PAP) is a secretory protein highly up-regulated during acute pancreatitis. PAP supposedly has a protective effect against inflammation. In this project we collaborated with R. DeLisle (Kansas) to investigate whether PAP is protective in a model of cystic fibrosis. Cystic fibrosis is associated with sporadic pancreatic inflammation and insufficiency. It was observed that these mice have much higher levels of PAP in the pancreas. We hypothesized that CF mice with acute pancreatitis would be protected compared with wildtype mice. Therefore, an acute pancreatitis was induced in CF mice. Despite high expression levels of several pancreatic stress proteins before and during acute pancreatitis, the CF mice exhibited no difference in the extent and severity of acute pancreatitis (Norkina et al., 2006, BMC Gastroent. 6, 16).

Achievements 2006

Scientific

- We demonstrated the presence of PGE_2 receptors in chronic pancreatic inflammation.
- We were able to demonstrate a synergistic effect of TNF and PGE_2 in primary pancreatic lobuli.
- Apoptosis is inhibited by alcohol and tissue injury is switched to a necrotic type of cell death. Publication in Am. J. Physiology.
- Grants awarded from the Swiss National Science Foundation and the Velux Foundation

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. Robert DeLisle, Anatomy and Cell Biology, University of Kansas School of Medicine, Kansas City, KS
- Prof. M. Bachem, Klinische Chemie, Universitätsklinikum, Ulm

Selected references:

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- Fortunato F, Deng X, Gates LK, McClain CJ, Bimmler D, Graf R, et al. Pancreatic Response to Endotoxin after Chronic Alcohol Exposure: Switch from Apoptosis to Necrosis? Am J Physiol Gastrointest Liver Physiol 2006, 90: G232-41.

2.2.4 **Surgical Skill Center**

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

PD Dr. med. Dieter Hahnloser

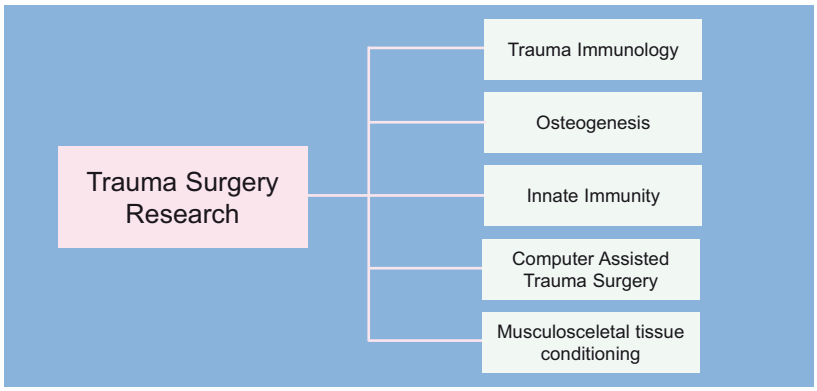
A competent surgeon should possess knowledge, judgment, experience and operative skills. Because of increasing time constraints, cost, and the increasing complexity of operations, the modern operating room is no longer the optimal learning environment. Surgical skills are clearly linked to the clinical outcome of surgical procedure, thus influencing patient's safety.

The Division of Visceral and Transplantation Surgery has started a project to build and run a surgical skills center in Zurich. Using computer-based virtual reality simulators trainees can practice standardized laparoscopic tasks repeatedly, with instant objective feedback or performance without jeopardizing patient's safety. Ongoing research will provide data for a standardized, validated curriculum for basic surgical skills training which will become required before operating on patients.

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

Decay of lysosomes during apoptosis in neutrophil granulocytes: An alternative to the caspase pathway?
PD Dr. med. Marius Keel MD, Dr. rer. nat. Luc Härter

Trauma Patients with severe, or multiple injuries are at high risk to develop posttraumatic complications which increase their late morbidity and mortality. In contrast to early mortality, the posttraumatic complications are promoted by the host immune response. Neutrophil Granulocytes (PMN) contribute to the pathogenesis of the systemic inflammatory response syndrome (SIRS), multiple organ dysfunction syndrome (MODS), sepsis and the multiple organ failure (MOF), by propagating and accelerating the pro-inflammatory immune response of the host. During SIRS and sepsis the spontaneous apoptosis in neutrophil granulocytes (PMN) is reduced, which leads to an increased survival and accumulation of PMN. The local accumulation of PMN at sites of inflammation contributes to a higher morbidity and mortality in these patients. The regulation mechanisms of spontaneous PMN apoptosis remain unclear. In previous investigations we could show that spontaneous PMN apoptosis was independent of caspase-3 activation and other proteases must be involved. Therefore the participation of other enzymes, especially lysosomal enzymes in regulation and execution of spontaneous PMN apoptosis was investigated in this study.
PMN isolated from septic patients and healthy volunteers were preincubated with Bafilomycin A_1 (10nM) for 1h and then stimulated with LPS (1µg/mL). Apoptosis was measured in FACS after FITC-Annexin-V and propidium iodide staining. Lysosomal integrity was measured in FACS with Rhodamine 6G (Rho6G) staining. Spontaneous apoptosis of PMN was significantly reduced in septic patients (57.3 ± 4.4% vs. 25.3 ± 6.9%, apoptotic cells) after 16h. LPS significantly reduced spontaneous PMN apoptosis (35.7 ± 3.7% vs. 14.8 ± 6.0%) and Bafilomycin A1 completely inhibited this effect (48.8 ± 3.7% vs. 20.3 ± 7.2%). Exclusion of Rho6G after 16h was significantly higher in PMN from healthy volunteers compared to septic patients (64.75 ± 3.3% vs. 34.3 ± 6.9%,

Rho6G negative cells). LPS significantly reduced Rho6G exclusion in both groups (36.3 ± 3.2% vs. 20.0 ± 3.9%) compared to untreated samples. Bafilomycin A_1 almost completely abolished the LPS effect (60.0 ± 4.7% vs. 28.6 ± 3.9%).

The decay of lysosomes which are filled with proteolytic enzymes contributes to the execution of spontaneous apoptosis and may represent an alternative to the caspase pathway.

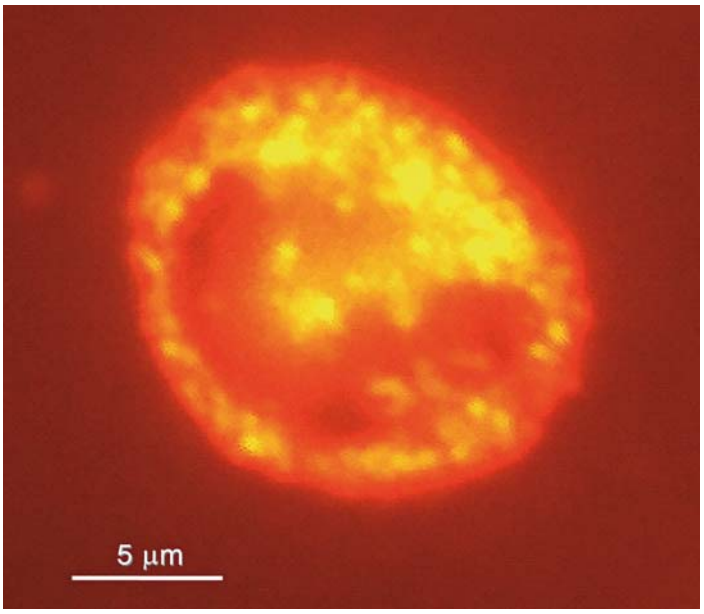


Figure 1
Native polymorphonuclear neutrophil granulocyte (PMN) stained with Rhodamin-6G under fluorescent light microscopy. Clearly visible are the multiple lysosomes and the negatively stained nucleus.

Achievements 2006

- M. Keel, Die Tamponade – meine sicherste Methode der Blutstillung. 5. Homburger Beckenkurs, Homburg 12-15.09.2006
- M. Keel, The Zürich Experience. F7-Trauma 1711 Investigators Meeting, Frankfurt 09.06.2006
- VAC Therapie in der Traumatologie. L. Labler, 9. Swiss Intensive Symposium SIS, Nottwil 07.03.2006
- Trauma Management und moderne Behandlungskonzepte bei Mehrfachverletzten. M. Keel, 9. Swiss Intensive Symposium SIS, Nottwil 07.03.2006
- M. Keel, Medizintechnik im täglichen Einsatz. Nationaler Tag der Technik, ETH, Zürich 30.11.2006
- M. Keel, Nekrotisierende Weichteilinfektionen, Fasziitis, Tetanus. Fortbildung Intensivmedizin, USZ, 16.10.2006
- M. Keel, Multilevel trauma care in Switzerland. 16th BOTA Symposium, Lüttich, Belgien 09.09.2006
- M. Keel, Versorgung schwerverletzter Patienten am USZ, Tag der offenen Tür, USZ 08.04.2006
- M. Keel, Damage Control-Konzept: Geschichte und Aktualität. Trauma Management beyond ATLS Teil III/IV, USZ, ZH 05.04.2006
- L. Labler, Resuscitation-Thorakotomie: Frustran oder Erfolgsgekrönt? Trauma Management beyond ATLS Teil III/IV, USZ, ZH 05.04.2006
- L. Labler, Nekrotisierende Fasziitis, Infektionen in der Gynäkologie, USZ, 23.11.2006
- Toll-like receptors on monocytes are altered during sepsis L. Härter, L. Mica, O. Trentz, M. Keel, ZKF, 5th Day of Clinical Research. 23-24.03.2006
- LPS prevents lysosomal decay during spontaneous apoptosis in neutrophil granulocytes. L. Mica, L. Härter, O.Trentz, M. Keel, ZKF, 5th Day of Clinical Research. 23-24.03.2006
- Protein S100; Marker for mild traumatic brain injury. L. Labler, SGKC Jahreskongress, St. Gallen 27-29. 09.2006
- Trauma death within the first 24 hours after admission to a tertiary trauma center: A retrospective analysis. E. Benninger, L. Labler, O. Trentz, M. Keel, 93. SGC Kongress, Lugano, 21-23.06.2006
- Increased survival of neutrophil granulocytes in VAC®-treated compared to Epigard®-treated wounds. L. Mica, L. Labler, O. Trentz, L. Härter and M. Keel, 93. SGC Kongress, Lugano, 21-23.06.2006
- LPS prevents lysosomal decay during spontaneous apoptosis in neutrophil granulocytes. L. Mica, L. Härter, O. Trentz, M. Keel, 93. SGC Kongress, Lugano, 21-23.06.2006
- Increased mortality in the polytraumatized elderly patient: a retrospective overview-study with 780 patients. T. Lustenberger, L. Mica, L. Labler, O. Trentz, M. Keel, 93. SGC Kongress, Lugano, 21-23.06.2006
- Cervical spine trauma of the elderly. TS. Vetter, L. Labler, O. Trentz, 7th Europ. Trauma Congress, Ljubljana, 14-17.05.2006
- Outcome of polytraumatized elderly patient: a retrospective overview-study of 780 patients. L. Mica, L. Labler, T. Lustenberger, O. Trentz, M. Keel,

7th Europ. Trauma Congress, Ljubljana, 14-17.05.2006

- Up-regulation of Toll-like receptors on monocytes during sepsis. L. Härter, L. Mica, O. Trentz, M. Keel, 7th Europ. Trauma Congress, Ljubljana, 14-17.05.2006
- Mild traumatic brain injury in the elderly patients. L. Labler, L. Mica, O. Trentz, H. G. Imhof, 7th Europ. Trauma Congress, Ljubljana, 14-17.05.2006
- Influence of V.A.C.-therapy on cytokines and growth factors in traumatic wounds. L. Labler, V.A.C ®. Therapie, 3-Länder Kongress, Nürnberg 28-29.04.2006

Collaborations:

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

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- Labler L, Keel M, Trentz O, Heinzelmann M. Wound conditioning by vacuum assisted closure (V.A.C.) in postoperative infections after dorsal spine surgery. Eur Spine J. 2006;15:1388-1396.
- Labler L, Mica L, Härter L, Trentz O, Keel M. Influence of V.A.C.-therapy on cytokines and growth factors in traumatic wounds. Zentralbl Chir. 2006;131:S62-67.
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- von Kanel R, Hepp U, Buddeberg C, Keel M, Mica L, Aschbacher K, Schnyder U. Altered blood coagulation in patients with posttraumatic stress disorder. Psychosom Med. 2006;68:598-604.
- Meier C, Keller IS, Pfiffner R, Labler L, Trentz O, Pfammatter T. Early experience with the retrievable OptEase vena cava filter in high-risk trauma patients. Eur J Vasc Endovasc Surg. 2006;32:589-595.
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2.3.2 **Osteogenesis Laboratory & Bone Research**

Dr. med. Alexander E. Handschin

Dr. med. Omana A. Trentz

Sonja Hemmi

PD Dr. med. Guido Wanner

Dr. med. A. Handschin, Dr. med. O.A. Trentz, S. Hemmi,
PD Dr. med. G. Wanner

Ectopic bone formation in vitro

Heterotopic ossification (HO) is the pathologic formation of bone in soft tissue. This process can occur after traumatic brain injury, fractures, and burns. The exact pathomechanism of HO is unknown but probably involves a disturbed bone formation process including osteoblast differentiation. However, to date little is known about the regulatory proteins and cytokines involved in HO formation. Leptin, known as the obesity gene, may regulate normal osteoblast function in vitro. An in vitro study was conducted to further analyse the pathomechanisms of HO, including a possible effect of leptin in the genesis and regulation of ectopic bone formation.
In both normal osteoblasts and cells isolated from heterotopic bone, leptin increased the formation of bone nodules in the histological analysis dose-dependent. Extracellular matrix mineralization (Ca-45 incorporation) was significantly increased with 100 -1000ng/ml leptin in both groups. This is the first study to analyse the effect of leptin on bone cells from heterotopic ossification. Similar to the in vitro behaviour of normal osteoblasts, cells from HO respond to leptin exposure with an increased mineralization of the extracellular matrix. In vitro, this response occurs during an earlier stage of cellular differentiation. This mechanism may be involved in the pathogenesis of ectopic bone formation in vivo.

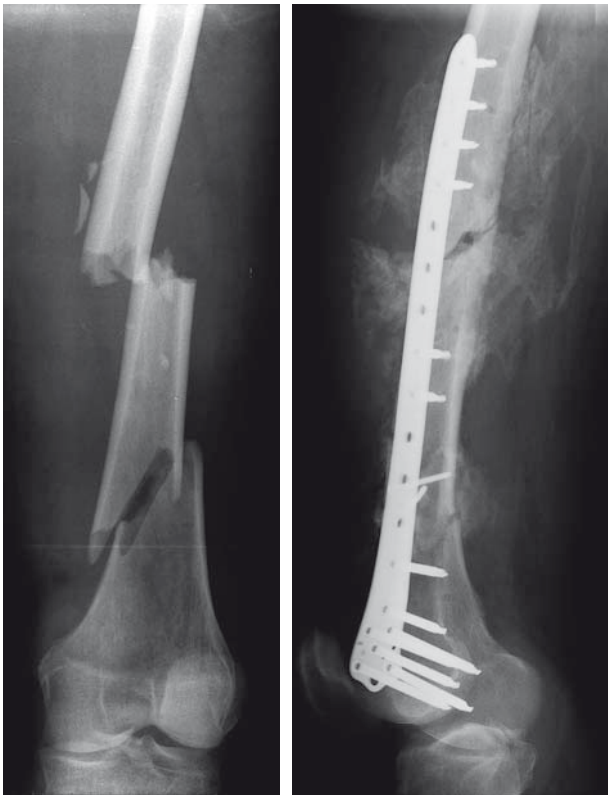


Figure 1: Ectopic bone formation in the femoral area following fracture and osteosynthesis.

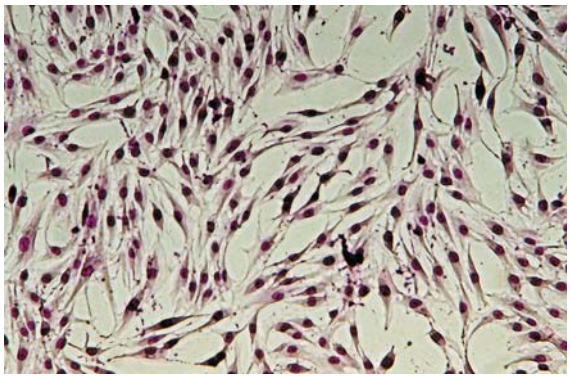


Figure 2: Cells from HO in vitro

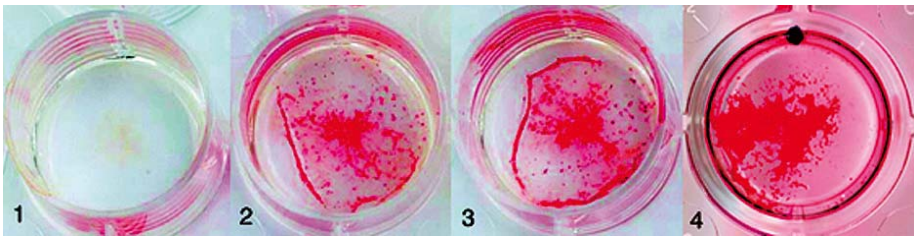


Figure 3: Increasing doses of leptin (0-1000ng/ml) induce a mineralization of the extracellular matrix in vitro.

Effect of Heparin on human osteoblasts in vitro

Heparin may cause adverse effects on bone formation following long-term application. The exact pathomechanism is unclear, but in vitro data suggest an impaired osteoblast function. The transcription axis of Cbfa-1 (Runx-2) and osteocalcin is crucial in maintaining an equilibrium of bone formation and resorption in vivo. We used a human osteoblast cell culture model to further investigate the effect of heparin (low-molecular-weight heparin, dalteparin) on the expression of these two regulators of osteoblast differentiation. At high doses, dalteparin caused a significant inhibition of both osteocalcin and Cbfa-1 expression in vitro. Our data support the hypothesis of a direct inhibition of osteoblast function underlying heparin osteoporosis.

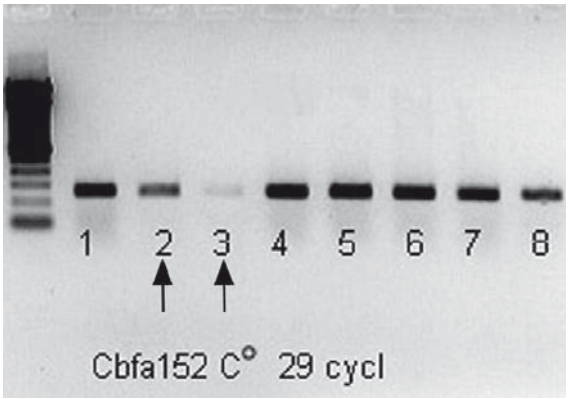


Figure 4: RT-PCR, Osteocalcin expression is inhibited by increasing doses of low molecular weight heparin (1-3, arrows, Fragmin). 4-8: control.

Achievements 2006

Oral presentation:

- Handschin AE, Egermann M, Wanner GA, Trentz O, Kock HJ, Trentz OA. Wirkung von niedermolekularem Heparin (Dalteparin) und Fondaparinux (Arixtra®) auf humane Osteoblasten in vitro. Chirurgisches Forum DGCH, Berlin 2006

Collaborations:

- Division of Plastic, Reconstructive and Hand Surgery, University Hospital of Zurich
- U.S. Department of Defense and Department of Veteran Affairs: Research project on posttraumatic heterotopic ossifications in soldiers during Iraq/ Afghanistan conflict.
- PD Dr. med. H.J. Kock, Unfallchirurgie, Hochtaunuskliniken, Bad Homburg
- Department of Plastic, Reconstructive and Aesthetic Surgery, Inselspital Bern, Dr. M. Constantinescu
- Department of Hand Surgery, Inselspital Bern, PD Dr. E. Voegelin

Selected references:

- Handschin AE, Trentz OA, Hemmi S, Wedler V, Trentz O, Wanner GA Leptin increases extracellular matrix mineralization of osteoblasts from heterotopic ossification and normal bone Ann Plast Surg, accepted Nov 18th, 2006, in press
- Handschin AE, Egermann M, Wanner GA, Trentz O, Kock HJ, Zund G, Trentz OA Cbfa-1 (Runx-2) and osteocalcin expression in heparin osteoporosis in vitro Clin Appl Thromb Hemost 2006, 12: 465-472
- Handschin AE, Egermann M, Wedler V, Trentz O, Hemmi S, Trentz OA A comparative analysis of phenotype expression in human osteoblasts from heterotopic ossification and normal bone. Langenbeck's Arch Surg 2006, 391: 376-382
- Fabry W, Trampenau C, Bettag C, Handschin AE, Lettgen B, Huber FX, Hillmeier J, Kock HJ Bacterial decontamination of surgical wounds treated with Lavasept® Int J Hyg Environ Health 2006, 209: 567-573

2.3.3 Innate Immunity Laboratory

PD Dr. med.
Michael
Heinzelmann

Dr. med.
Herbert Bosshart

Lipopolysaccharide-Induced Activation of Human Peripheral Blood Monocytes

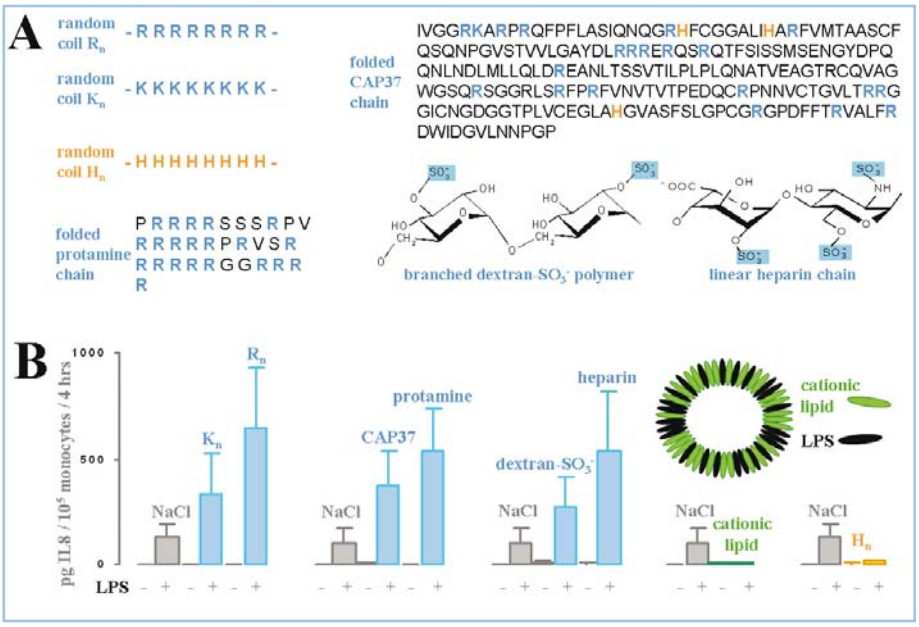
Michael Heinzelmann & Herbert Bosshart

The ensuing immune response evoked by invading bacteria does not require previous exposure to the pathogen. As opposed to acquired immunity, innate immune responses are based on the recognition of conserved molecular structures such as Gram-negative lipid A or Gram-positive peptidoglycan and lipoteichoic acid.

In some cases, bacterial or fungal infections lead to a dysregulated innate immune response. The result of such a response gone awry is twofold. First, the infection cannot be resolved. Second and more importantly, the inadequate immune response causes damage to the host. Early clinical signs of such ensuing damage are hyper- or hypothermia, a racing heart beat, fast breathing, and a white blood cell count well above the normal range. Clinicians use the term sepsis to describe this condition. While the pathogenesis of the sepsis syndrome is extremely complex with many different clinical endpoints, i.e. hypotension, hyperglycemia, lactic acidosis or disseminated intravascular coagulation (leading to bleeding or thrombosis), unabated progression of the condition invariably results in respiratory and renal failure and an ever increasing oxygen debt, which eventually leads to progressive organ failure and death.

Incidence rates and, hence, the global public health impact of the sepsis syndrome remain relatively low. Severe forms of sepsis occur in only two or three cases per 1000 hospital admissions. However, with mortality rates of 20 % for severe forms of sepsis, 50 % for patients with septic shock and well above 70 % for septic conditions associated with multiple organ failure, it is not surprising that enormous research efforts are being undertaken to develop novel anti-sepsis drugs. Although our understanding of the molecular mechanisms involved in the pathogenesis of the sepsis syndrome has grown remarkably in recent years, this wealth of knowledge has not had the expected impact on the reduction of mortality rates.

Over the past few years, we identified a number of diverse molecular structures with a capacity to either enhance or inhibit monocyte activation induced by Gram-negative endotoxin. Examples are shown in the figure below. The strategy employed could lead to the development of novel drugs that safely and effectively neutralize Gram-negative endotoxin in vivo.



Modulation of LPS-induced monocyte activation by polypeptides, polysaccharides and lipids. (A) Structural features of chemically divers LPS-modifying substances. LPS signals are enhanced by random coil polymers of L-arginine (R_n) and L-lysine (K_n), and blunted by poly-L-histidine (H_n). Arginine-rich salmon protamine and human CAP37 enhance LPS responses. Depicted is the protamine sequence from chum salmon (Oncorhynchus keta) and the sequence for human CAP37. Sulfated carbohydrates, e.g. branched dextran sulfate polymers or linear heparin chains, enhance LPS signals. Representative disaccharide structures are shown. O- and N-linked sulfate groups are highlighted by shaded boxes. (B) IL8 release in human whole blood, treated with or without LPS (-/+), in the presence or absence of LPS-modifying substances. The proposed association of LPS with cationic lipids in the form of a mixed micelle (vesicular structure) is shown. Bars represent picogram amounts (pg) of IL8, secreted from 10^5 peripheral blood monocytes during a 4 hour incubation period at 37°C. Error bars indicate one standard deviation (n = 4 healthy donors). In the presence of LPS (+), all test samples show significant differences compared to the corresponding saline (NaCl) controls ($p < 0.05$). LPS: 10 ng/ml; random coil polypeptides (R_n, K_n, H_n): 10 micrograms/ml; CAP37: 25 micrograms/ml; heparin: 100 IU/ml; protamine: 100 IU/ml; cationic lipids (Lipofecatmine™ 2000, Invitrogen): 10 microliters/ml.

Achievements 2006

Published Work

- Bosshart H, Heinzelmann M. (2007). Targeting bacterial endotoxin: two sides of a coin. Ann N Y Acad Sci, 1096: 1-17.

Scientific Meetings

- Bosshart H, Heinzelmann M. Targeting bacterial endotoxin: two sides of a coin.
  Cell Signaling World - Signal Transduction Pathways as Therapeutic Targets, January 25-28, 2006, Luxembourg.

Collaborations:

- Hans Flodgaard, Leukotech, Fruebjergvej 3, Box 8, 2100 Copenhagen - Denmark
- Jean-Marc Herbert, Cardiovascular and Thrombosis Research Department, Sanofi Synthelabo, 195 route d'Espagne , 31036 Toulouse CEDEX - France
- 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

Selected references:

- Bosshart H, Heinzelmann M. (2007). Targeting bacterial endotoxin: two sides of a coin. Ann N Y Acad Sci, 1096: 1-17.
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- Bosshart H, Heinzelmann M (2004). Lipopolysaccharide-mediated cell activation without rapid mobilization of cytosolic free calcium. Mol Immunol, 41:1023-8.
- Bosshart H, Heinzelmann M (2004). Human neutrophil-derived CAP37 inhibits lipopolysaccharide-induced activation in murine peritoneal macrophages. Immunol Lett, 94:175-82.
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- Bosshart H, Heinzelmann M (2003). Endotoxin-neutralizing effects of histidine-rich peptides. FEBS Lett, 553:135-40.
- Bosshart H, Heinzelmann M (2002). Arginine-rich cationic polypeptides amplify lipopolysaccharide-induced monocyte activation. Infect Immun, 70:6904-10.

2.3.4 Computer Assisted Trauma Surgery (CATS)

PD Dr. med.
Peter Messmer

Dr. med.
Felix Matthews

Dipl. Ing. FH
Adrian John

Dipl. Inf. Ing. ETH
Adrian Egli

Dr. med., med. dent.
Heinz-Theo Lübbers

med. pract.
Vladislav Raykov

PD Dr. med. Peter Messmer, PD Dr. med. Guido A. Wanner,
Dr. med. Felix Matthews, Dipl. Ing. FH Adrian John, Dipl. Inf. Ing. ETH Adrian Egli,
Med pract. Vladislav Raykov, Dr. med., med. dent. Heinz-Theo Lübbers

Navigation is mainly introduced in complex maxillo-facial surgery for reconstruction after tumor resection at the head but also for correction of posttraumatic malalignements. In one case the system was used for orthognatic surgery. All together twenty cases have been operated so far using navigation. However it is still not a routine, rather a way of discovering and testing the value and limitations of computer assisted surgery in the field. A new concept for navigated dental implantation is under development. In the laboratory we performed a comparative study of different registration methods for navigation in maxillofacial surgery. The work is submitted for publication.

Together with AO-International (AOI), BrainLab and other partners from the Computer-assisted Surgery Expert Group of the AO (University of Ulm, BG Klinikum Ludwigshafen, MH Hannover) teaching modules with workshops, videos and posters for navigation in fracture treatment and osteotomies were developed.

A main focus of our research group is now technology integration and development of advanced planning and handling tools. With the i3db project we develop a platform for integration of in the hospital already existing but also new software solutions for different applications such as Clinic Information Systems, PACS, material storage -, documentation - and accounting-software. We developed together with our partner (Business Innovation Centre, BIC, Siemens Schweiz) a database of implants, instruments and information, which is enhanced with an interactive 3D planning tool for fracture treatment. The partner for image processing is Siemens Med, Erlangen, Germany, the one for implants is Synthes, Solothurn, Switzerland. Our tool for haptic real time osteosynthesis planning was presented at the booth of SensGraphicsR during the MMVR 15 conference in California and at the World Haptics conference in Japan. The project is supported by KTI/CTI (Förderagentur des Bundes für Innovation und Technik).

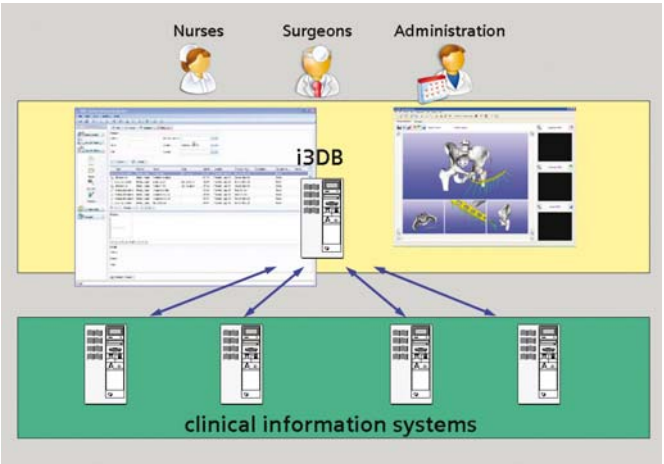

Figure 1: i3db serves as a platform integrating different software systems with the goal of optimization of workflows (see also PULS 2/06, p19-21, Personalzeitschrift USZ).

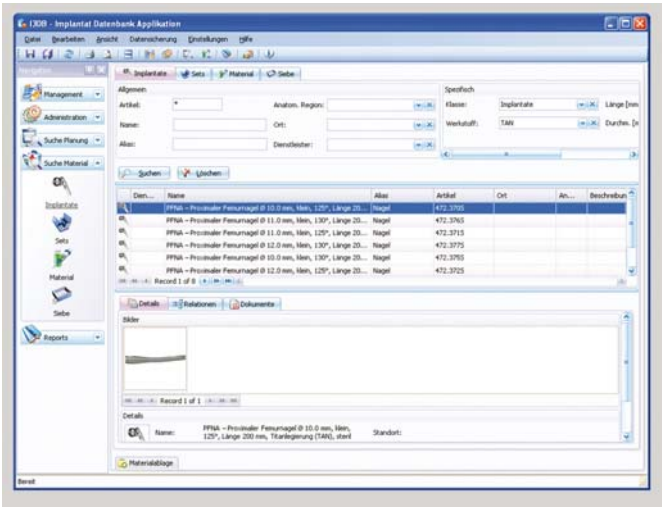

Figure 2: Implant database as a function of the workflow database. This tool is used during preoperative planning by the surgeon and preparation of the operation by the OR nurse.

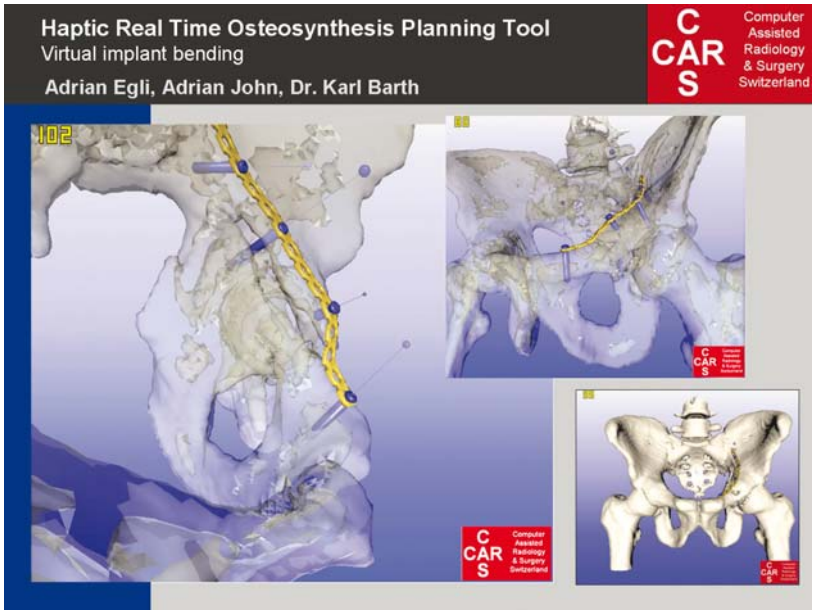

Figure 3: Virtual 3D planning as a further function of i3db. Patient data are directely imported from Clinic Information System (CIS) and Radiology Information System (RIS). Implant data are stored in the implant-database. With a haptic feedback device the surgeon can feel the virtual model of the fractured pelvis and easily determine where he wants to place the plate. By transformation of the 3D coordinates of the surface the plate is shaped. Transparency of the model helps the surgeon to avoid placing the screws into the joint. Several measuring functions inform the surgeon already preoperatively about the implants he will need. All this planning information will be available on the intranet for the nurse to prepare her- or himself and the operation saving time and material during the operation.

Achievements 2006

- Continuation of cooperation with industrial partners: Siemens, Synthes, Zimmer, BrainLab, SenseGraphics
- Cooperation with CARCAS Basel continued
- i3db workflow project: prototype developed, first evaluation started
- Maxillo-facial navigation in clinical testing

Collaborations:

- Prof. Grätz, Dr. Dr. J.A. Obwegeser, Dr. H. Lübbers, Klinik und Poliklinik für Kiefer- und Gesichtschirurgie, Universitätsspital Zürich
- CARCAS, Research Group for Computer Assisted Radiology and Surgery of the University Hospitals of Basel and Zurich
- Dr. D. Holzmann, Klinik für Ohren-, Nasen-, Hals- und Gesichtschirurgie, Universitätsspital Zürich
- Expert Group Computer Assisted Surgery of the AO Foundation with the following partners:
  - Prof. C. Krettek, Unfallchirurgische Klinik, Medizinische Hochschule Hannover MHH
  - Prof. F. Gebhard. Abteilung für Unfallchirurgie, Hand- und Wiederherstellungs-chirurgie, Universitätsklinikum Ulm
  - Prof. J. Alonso, Orthopaedic Trauma Service, University of Birmingham, Alabama
  - PD Dr. P.A. Grützner, Klinik für Unfall und Wiederherstellungschirurgie, BG Unfallklinik Ludwigshafen
  - Prof. R. Kikinis, Surgical Planning Laboratory, Brigham and Women's Hospital, Harvard Medical School, Boston, USA
- AO Development Institut, Davos
- University of Applied Sciences Bern/Biel
- Siemens Med, Erlangen, Deutschland
- Siemens Schweiz, Zürich Altstetten
- BrainLab, Heimstetten, Deutschland
- Synthes, Solothurn
- Kommission Innovation und Technik, Bundesamt für Berufsbildung und Technologie
- Eidgen. Stipendienkommission, Staatssekretariat für Bildung und Forschung

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- Matthews F, Trentz O, Jacob AL, Kikinis R, Jupiter JB, Messmer P.: Protrusion of hardware impairs forearm rotation after olecranon fixation. A report of two cases. J Bone Joint Surg Am. 2007 Mar;89(3):638-42.
- Messmer P, Matthews F, Jacob AL, Kikinis R, Regazzoni P, Noser H. A CT Database for Research, Development and Education: Concept and Potential. J Digit Imaging. 2007 Mar;20(1):17-22.
- P. Messmer, F. Matthews, C. Wullschleger, R. Hügli, P. Regazzoni, A.L. Jacob: Image Fusion for Intraoperative Control of Axis in Long Bone Fracture Treatment. Eur J Trauma, No 6, 2006: p 555-561

2.3.5 Musculosceletal tissue conditioning

PD Dr. med.
Guido Wanner

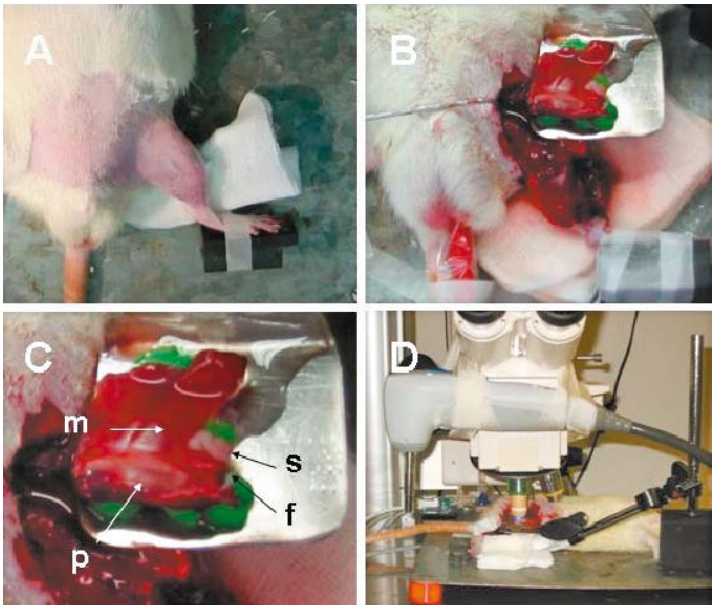
Dr. med.
Claudio Contaldo

Ahmed Elsherbiny
Wiss. Mitarbeiter

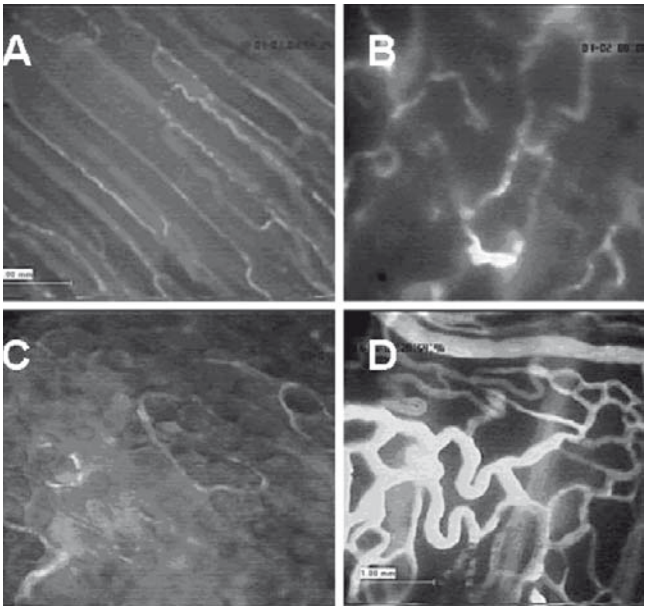
Andrea Schleh
Wiss. Mitarbeiterin

Erythropoietin protects axial pattern osteomyocutaneous flaps from reperfusion injury: An intravital microscopy study in sprague dawley rats
Dr. med. Claudio Contaldo; Ahmed Elsherbiny, Schleh Andrea,
PD Dr. med. Guido A. Wanner

Flap surgery is associated with surgical trauma and transfer-related ischemia/reperfusion (I/R) injury, potentially resulting in flap failure with deleterious consequences for the patient. Originally, stimulation of erythropoiesis was thought to be the only physiological function of Erythropoietin (EPO). However, the recently recognized pleiotropic and nonhematopoietic properties of this glycoprotein hormone are currently under intense investigation. The aim of this study was to determine whether pretreatment with EPO improves microvascular perfusion in prolonged ischemic pedicled osteomyocutaneous flaps. In the present study pedicled osteomyocutaneous flaps in Sprague Dawley rats were subjected to a primary period of one hour of ischemia followed by one hour of reperfusion to mimic the time required for microvascular anastomosis. To mimic a postoperative vessel occlusion a secondary period of two hours of ischemia followed by two hours of reperfusion was performed thereafter. Animals received Recormon ® 5000U/kg body weight i.p. 30min before primary ischemia (n=6). Vehicle treated animals (n=6) served as controls. The microcirculation of the muscle, skin, subcutaneous and periostal tissue of the flap was analysed quantitatively using epi-illumination intravital fluorescence microscopy. Tissue samples were analyzed for expression of EPO-receptor (EPO-R) and eNOS. Prolonged ischemia followed by reperfusion provoked a substantial loss of nutritive tissue perfusion in the muscle, subcutaneous and periostal tissue ($P < 0.05$ versus baseline), as indicated by the reduced functional capillary density and reduced volumetric capillary blood flow, accompanied by a massive accumulation of activated leukocytes ($P < 0.05$ versus baseline). EPO pretreatment improved tissue microhemodynamics and attenuated the inflammatory response (both $P < 0.05$ versus control). Immunohistochemical tissue analysis revealed an upregulation of EPO-receptor and eNOS after EPO pretreatment. The principal findings of the present study are that EPO pretreatment is capable of attenuating the microcirculatory dysfunctions and leukocyte-endothelial cell interactions after prolonged ischemia and reperfusion in pedicled osteomyocutanous flaps of the rat. Our study suggests that in the presence of hypoxia, EPO pretreatment may exert its protection through an enhanced bioavailability of nitric oxide. EPO may represent a novel therapeutic option for the plastic surgeon in situations, which are characterized by tissue hypoxia due to microhemodynamic derangements.



The saphenous pedicled osteomyocutaneous composite flap model in the rat. After shaving the left hind limb, a circular skin island flap is designed (*A*). After elevation, the osteomyocutaneous composite flap is mounted on a specially designed stage (*B*) for visualization of the microcirculation with epi-illumination intravital microscopy (*D*). The flap (*C*) consists of tibial bone with overlying periost (*p*), muscle (*m*), subcutaneous fat (*f*) and skin (*s*).



Intravital fluorescence microscopy (contrast enhancement by 5% FITC-dextran 150,000) of the four different tissues in pedicled osteomyocutaneous flaps of the rat. Visualization of the microcirculation of muscle capillaries (*A*), shows the parallel arrangement with virtually no intercapillary connections. In contrast the skin (*B*), and the subcutaneous tissue (*C*) reveal a mesh-like arrangement of capillaries. The periost-al tissue (*D*) shows the widest diameters, when visualized near to the major supplying and draining vessels. Magnification x900.

Collaborations:

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

Selected references:

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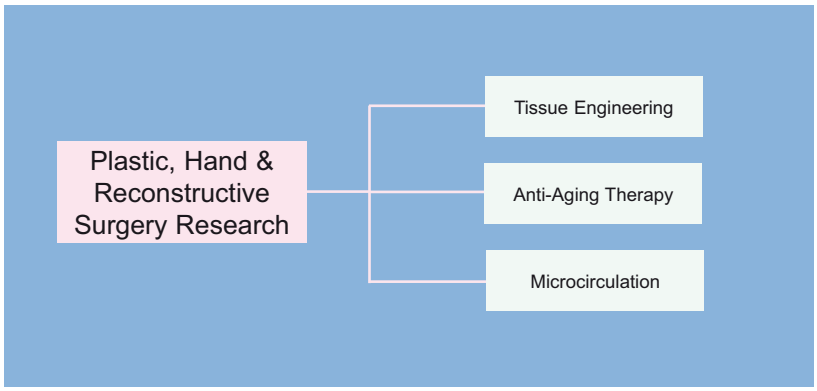
- Harder Y, Contaldo C, Erni D.Preconditioning with monophosphoryl lipid A improves survival of critically ischemic tissue. Anesth Analg. 2005

2.4 Plastic, Hand & Reconstructive Surgery Research

Prof. Dr. med.
Pietro Giovanoli,

Dr. med.
Maurizio Calcagni



2.4.1 Tissue Engineering

Dr. med.
Volker Wedler

Dr. med.
Claudio Contaldo

Dr. med.
Christian Köhler

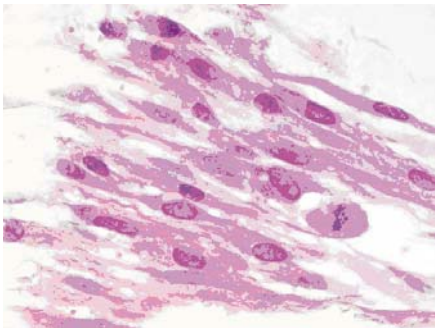
Manfred Welti

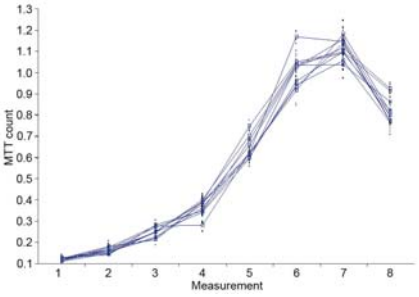
The regenerative capacity of cartilage is known to be very poor and is therefore a major problem in several diseases with severe cartilage loss - such as rheumatoid arthritis - traumatic articular defects and cartilage defects secondary to trauma or tumorsurgery. The reconstruction of a destroyed joint 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.

Impact of x-rays on human chondrocytes in the area of tissue engineering
C. Koehler, A. D. Niederbichler, J. Hellermann, B. Bode, J. Roos, M. Welti, V. Wedler

A significant logistic factor as to the successful clinical application of the autologous tissue engineering concept is efficient transportation of the donor cells to tissue processing facilities requiring air transportation necessitating x-ray safety checks. This study investigates how cartilage cells react to x-rays in quality and quantity. Special attention is paid to transillumination controls at airports.
Chondrocytes of a human joint were isolated and expanded in culture flasks using DMEM with FCS, gentamicin and amphotericin B. Cell cultures were incubated for 3 weeks and exposed to different doses and time periods of radiation. Subsequently, quantitative tests as to cell proliferation (MTT), qualitive tests as to protein production (GAG) and histological evaluations were performed. For prediction of cell growth, a new mathematical model was used.
The test showed that after irradiation of chondrocytes with different doses, no significant differences occurred compared with the control group. This applies to both exposure to luggage transillumination as well as exposure to radiation doses used clinically.

Any damage affecting cell growth or quality could not be observed in our study. However, information about damage of cell DNA remains incomplete. Due to our results the growth rate is predictable as a function over time.



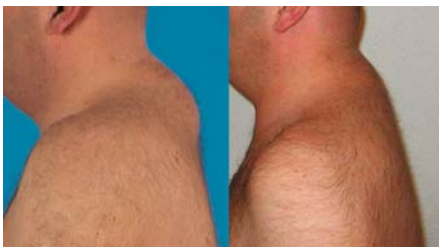


2.4.2 Anti Aging

Dr. med.
Christian Köhler

Suction lipoplasty: evaluation of complications and patients satisfaction
C. Koehler, M. Farshad, W. Kuenzi, V. Wedler

Liposuction is one of the most common aesthetic procedure used in plastic surgery. Reports are available on the results, the probable complications, and the feedback of patients. However, systematic studies dealing with these aspects using reliable large enough data is still needed.
The data comprised 116 procedures during a 6-years period up to 2005. The data were processed and categories of results were formed. Furthermore, a follow up examination and a survey on the feed back of patients was carried out.
Significant differences were identified in indications, results and complications. The follow-up examinations and the survey showed satisfying results. In the majority of all cases surgeons were satisfied with the operations.
In conclusion, if conducted by qualified surgeons in appropriate surgical conditions and postoperative care possibilities, liposuction may be considered as a reliable surgical procedure. The success of this procedure depends, however, on suitable infrastructure and operative competence.



2.4.2 Microcirculation

Dr. med.
Claudio Contaldo

PD Dr. med.
Guido Wanner

Ahmed Elsherbiny
Wiss. Mitarbeiter

Human recombinant erythropoietin protects the striated muscle microcirculation of the dorsal skinfold from postischemic injury in mice
C. Contaldo, C. Meier, A. Elsherbiny, O. Trentz, M. Menger, G.A. Wanner

Erythropoietin (EPO) has been proposed as a novel cytoprotectant in ischemia/reperfusion (I/R) of the brain, heart and kidney. There is no information, however, whether EPO exerts its protection by prevention of postischemic microcirculatory deteriorations. Here, we investigated the effect of EPO on I/R-induced microcirculatory dysfunctions. Using the mouse dorsal skinfold chamber preparation, nutritive microcirculation and leukocyte-endothelial cell interaction were studied in striated muscle of the dorsal skinfold by in vivo fluorescence microscopy before 3h ischemia and during 5 days reperfusion. Animals were pretreated with EPO (5000U/kg body weight) 1h or 24h before ischemia. Vehicle-treated I/R animals served as controls. Additional animals underwent sham operation only or received EPO pretreatment without I/R. I/R provoked a significant ($p<0.05$) reduction of functional capillary density, an increase of microvascular permeability, and an enhance-ment of venular leukocyte-endothelial cell interaction during early reperfusion. This was associated with a pronounced ($p<0.05$) arteriolar constriction and diminution of blood flow during late reperfusion. Pretreatment with EPO induced EPO-receptor and eNOS expression at 6h of reperfusion ($p<0.05$). In parallel, EPO significantly ($p<0.05$) reduced capillary perfusion failure and microvascular hyperpermeability during early reperfusion, and arteriolar constriction and flow reduction during the later reperfusion phase. EPO pretreatment further substantially ($p<0.05$) diminished I/R-induced leukocytic inflammation by reducing the number of rolling and firmly adhering leukocytes in postcapillary venules. Of interest, EPO, in particular if applied 1h before ischemia, additionally induced angiogenic budding and sprouting at day 1 and 3, and formation of new capillary networks at day 5 of reperfusion.
Thus, our study demonstrates for the first time that EPO effectively attenuates I/R injury by preserving nutritive perfusion, reducing leukocytic inflammation and inducing new vessel formation.

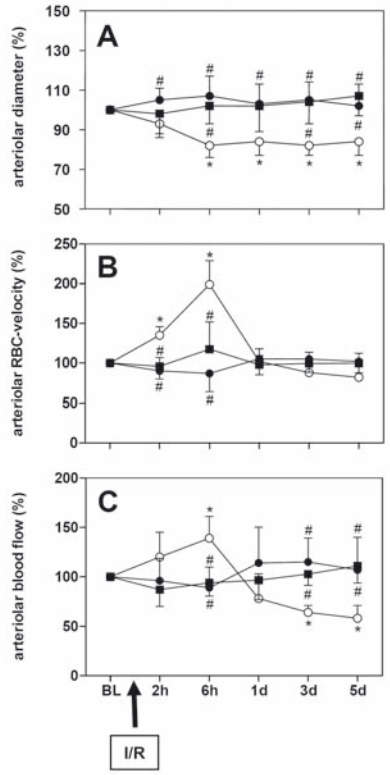


Fig 1. Arteriolar diameters (A), RBC velocity (B) and blood flow (C) (given in percent of baseline) in vehicle-treated I/R animals (○) and I/R animals pretreated with EPO 1h (●) or 24h (■) before induction of ischemia. Analyses were performed at baseline (BL) and at different time points during a 5-day postischemic observation period. The arrow indicates induc-tion of 3hours of ischemia and onset of reperfusion (I/R). Note the arteriolar constriction and reduction of arteriolar blood flow after I/R, and the prevention of postischemic deterioration of arteriolar blood perfusion after EPO pretreatment. Data are mean ± SD. *P < 0.05 vs BL; #P < 0.05 vs vehicle-treated I/R animals at corresponding time points.

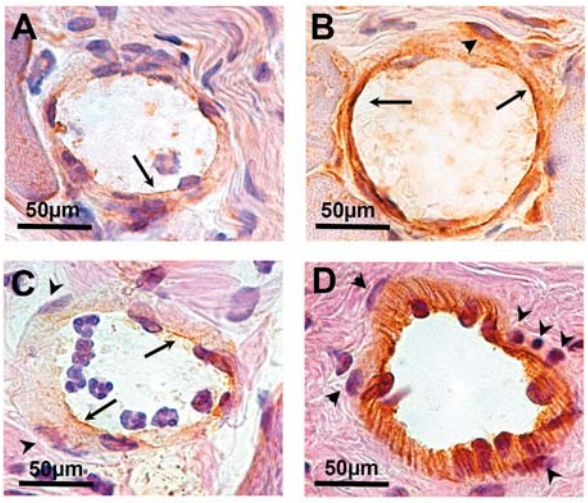
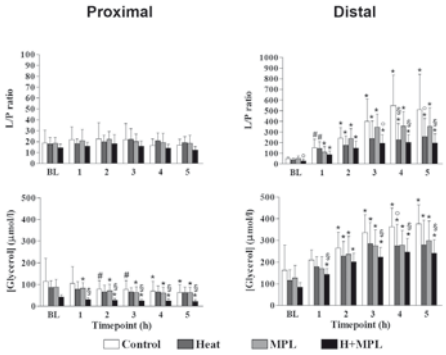


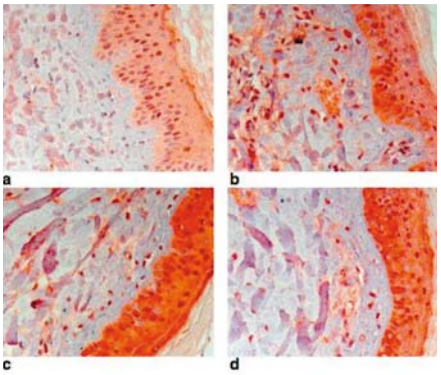
Fig. 2 Immunohistological demonstration of EPO-receptor (A, B) and eNOS (C, D) expression (indicated by brown staining) of endothelial cells (arrows) in cross sections of mouse striated muscle arterioles after 3h of ischemia and 6h of reperfusion. The normal expression of eNOS and EPO-R in sham-operated animals is shown in (A, C). Note the strong expression of EPO-receptor (B) and eNOS (D) after EPO pretreatment 1h before ischemia.

The influence of local and systemic preconditioning on oxygenation, metabolism and survival in critically ischaemic skin flaps in pigs
Claudio Contaldo

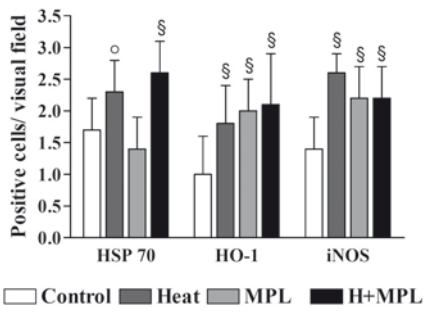
Stress proteins represent a group of highly conserved intracellular proteins that provide adaptation against cellular stress. The present study aims to elucidate the stress protein-mediated effects of local hyperthermia and systemic administration of monophosphoryl lipid A (MPL) on the oxygenation, metabolism and survival in bilateral porcine random pattern buttock flaps. Preconditioning was achieved 24 h prior to surgery by applying a heating blanket on the operative site (n=5), by intravenous administration of MPL at a dosage of 35 µg/kg body weight (n=5) or by combining the two (n=5). The flaps were monitored with laser Doppler flowmetry, polarographic microprobes and microdialysis until 5h postoperatively. Semiquantitative immunohistochemistry was performed for heat shock protein 70 (HSP70), heat shock protein 32 (also termed heme oxygenase-1, HO-1), and inducible nitrc oxide synthase (iNOS). The administration of MPL increased the impaired microcirculatory blood flow in the proximal part of the flap and partial oxygen tension in the the distal part by ~100% each (both $P < 0.05$), whereas both variables remained virtually unaffected by local heat preconditioning. Lactate/pyruvate (L/P) ratio and glycerol concentration (representing cell membrane desintegration) in the distal part of the flap gradually increased to values of ~500 mmol/l and ~350 µmol/l, respectively (both $P < 0.01$), which was substantially attenuated by heat application ($P < 0.01$ for L/P ratio and $P < 0.05$ for glycerol) and combined preconditioning ($P < 0.01$ for both variables), whereas the effect of MPL was less marked (ns). Flap survival was increased from 56% (untreated animals) to 65% after MPL (ns), 71% after heat application ($P < 0.05$) and 78% after both methods of preconditioning ($P< 0.01$). iNOS and HO-1 were upregulated after each method of preconditioning ($P < 0.05$), whereas augmented HSP70 staining was only observed after heat application ($P < 0.05$). We conclude that local hyperthermia is more effective in preventing from flap necrosis than systemic MPL administration because of enhancing the cellular tolerance to hypoxic stress, which is possibly mediated by HSP70, whereas some bene-fit may be obtained with MPL due to iNOS and HO-1-mediated improvement in tissue oxygenation.



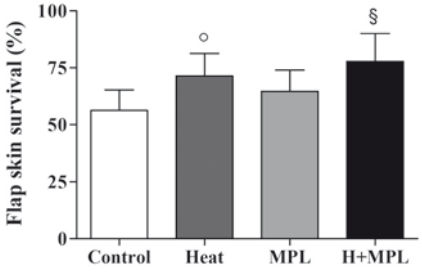
Tissue lactate to pyruvate ratio and glycerol concentrations measured by microdialysis in the proximal and distal flap skin in control animals and after preconditioning with heat (**Heat**), monophoshoryl lipid A (**MPL**) or both (**H+MPL**). The values represent mean ± SD. # P<0.05, *P<0.01 vs. baseline (BL); º P<0.05, §P<0.01 vs. Control.



Immunohistochemical staining for HO-1 in the distal flap tissue before surgery in con-trol animals (a) and after preconditioning with either heat (b), monophoshoryl lipid A (c) or both (d). Magnification x200; positively stained cells are auburn.



Density of cells stained for HSP70, HO-1 and iNOS in the distal flap tissue before sur-gery in control animals and after preconditioning with heat (**Heat**), monophoshoryl lipid A (**MPL**) or both (**H+MPL**). The values represent mean ± SD. º P<0.05, §P<0.01 vs. Control.



Flap skin survival in control animals and after preconditioning with heat (**Heat**), mono-phoshoryl lipid A (**MPL**) or both (**H+MPL**). The values represent mean ± SD. º P<0.05, §P<0.01 vs. Control.

Achievements 2006

Talks

- Contaldo Claudio:EURAPS-Meeting May 2006, Istanbul (European association of plastic surgeons of scientists and plastic surgeons); Effects of EPO on experimental hamster flap: doping for the microvasculature?
- Contaldo Claudio:ECSAPS-Meeting September 2006, London (European congress of scientists and plastic surgeons); Erythropoietin: a cytokine for reconstructive surgeons.
- Contaldo Claudio: 70. DGU-Tagung Oktober 2006, Berlin (Deutsche Gesellschaft für Unfallchirurgie); Effekte von EPO auf die Mikrozirkulation von kritisch perfundierten Experimentallappen.

Dissertationen

- "Effects of Human Recombinant Erythropoietin on Microhemodynamics in Critically Ischemic Hamster Skin." Arbeit vorgelegt von Herrn Ahmed Elsherbiny, unter der Leitung von Dr. med. C. Contaldo und PD Dr. med. G.A. Wanner, genehmigt März 2007 von der Medizinischen Fakultät der Universität Zürich, auf Antrag von Prof. Dr. med. O. Trentz.

Collaborations:

- Prof. Dr. med. Michael D. Menger, Dr. med. Yves Harder, Institut für Experimentelle Chirurgie, Universitätsklinikum, Homburg/ Saar, Deutschland
- Prof. Dr. med. Otmar Trentz, PD Dr. Guido A. Wanner, Dr. Christoph Meier, Klinik für Unfallchirurgie, Universitätsspital Zürich
- Prof. Dr. med. Andrej Banic, Prof. Dr. med. Dominique Erni, Dr. med. Jan Plock, Klinik für Plastische, Rekonstruktive und Aesthetische Chirurgie, Prof Dr. med. S.M. Jakob, ESI, Inselspital Bern

Selected references:

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- Contaldo C, Harder Y, Plock J, Banic A, Jakob SM, Erni D.The influence of local and systemic preconditioning on oxygenation, metabolism and survival in critically ischaemic skin flaps in pigs. J Plast Reconstr Aestetic Surg 2007
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- Plock J, Contaldo C, von Lüdinghausen M Extraocular eye muscles in human fetuses with craniofacial malfor-mations: Anatomical findings and clinical relevance. Clin Anat. 2006
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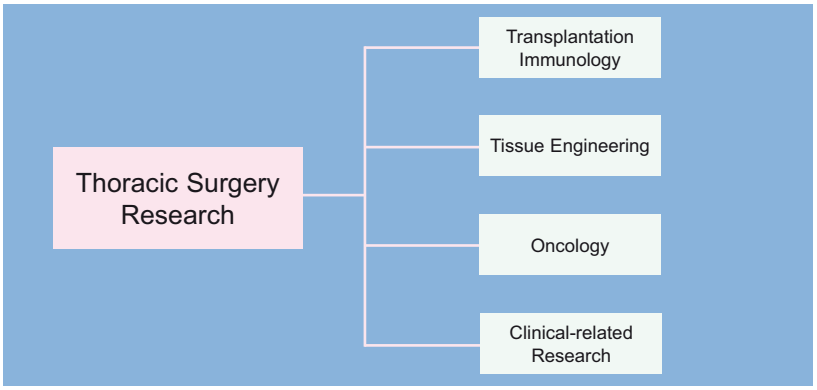
2.5 Thoracic Surgery Research

Dr. med.
Sven Hillinger

Prof. Dr. med.
Walter Weder



2.5.1 Transplantation Immunology

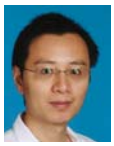
PD Dr. med.
Stephan Korom

Dr. med.
Sven Hillinger

PD Dr. med.
Ilhan Inci

Dr. med.
Florian Jung

Dr. med.
Markus Cardell



Dr. med.
Wei Zhai

Dr. rer. nat.
Stephan Arni

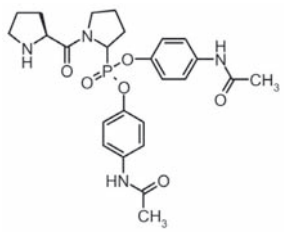
Dr. med.
Brigit Oberreiter

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

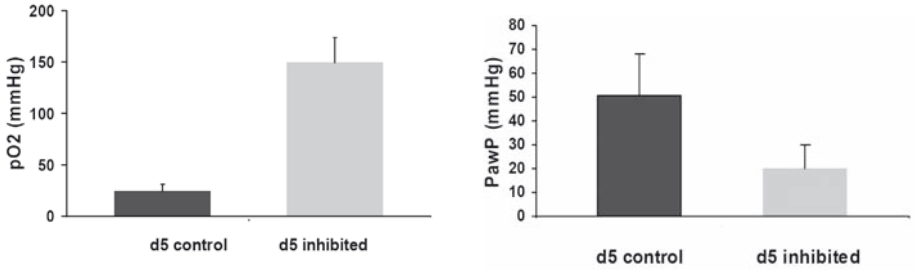
CD26/dipeptidylpeptidase IV-targeted therapy of acute lung rejection in rats
F.Jung, W.Zhai, M.Cardell, I.Inci, S.Arni, S.Korom

CD26 is a T-cell co-stimulator, and interacts with adenosine deaminase, human immunodeficiency virus (HIV) Tat-1 protein and extracellular matrix. It possesses dipeptidylpeptidase IV (DPP IV) catalytic activity, which is linked to its co-stimulatory efficacy. We investigated the effect of specific DPP IV systemic activity inhibition on acute pulmonary rejection.
Rat single-lung transplantation (Tx) was performed (LBNF1/LEW donor/recipient) in two groups (n = 12). Group I (n = 6) received daily treatment with a Pro-Pro-diphenylphosphonate derivative (AB197), and Group II served as an untreated control. At Day 5 post-Tx, ventilatory parameters, cytotoxicity and mixed lymphocyte reaction were analyzed and staining for ISHLT rejection grade and proliferating cell nuclear antigen (PCNA) was performed.
Treatment with AB192 abrogated acute rejection and preserved pulmonary function up to Day 5 post-Tx for PO2 (Group II: 24.9 +/- 6.9 mm Hg; Group I: 149.5 +/- 24.3 mm Hg; $p < 0.001$), PCO2 (Group II: 53.3 +/- 13.6 mm Hg; Group I: 39.0 +/- 9.8 mm Hg; $p < 0.05$) and peak airway pressure (Group II: 50.7 +/- 17.2 mm Hg; Group I: 20.2 +/- 10.0 mm Hg; $p < 0.01$). Controls showed moderate/severe rejection (ISHLT Grade A2 or 3), grafts from inhibited hosts revealed no/mild rejection (Grade A0 to 2: Group II: 2.8 +/- 0.3; Group I: 1.25 +/- 1.0; $p < 0.005$). Proliferating cell nuclear antigen (PCNA) staining of rejection-associated cellular infiltrates showed a significant reduction in positivity in perivascular infiltrates (34 +/- 11.5%; $p < 0.05$) and bronchial surface epithelium (31.7 +/- 10.6%; $p < 0.05$) in Group I vs Group II (55.9 +/- 8.4% and 57.2 +/- 4.5%).

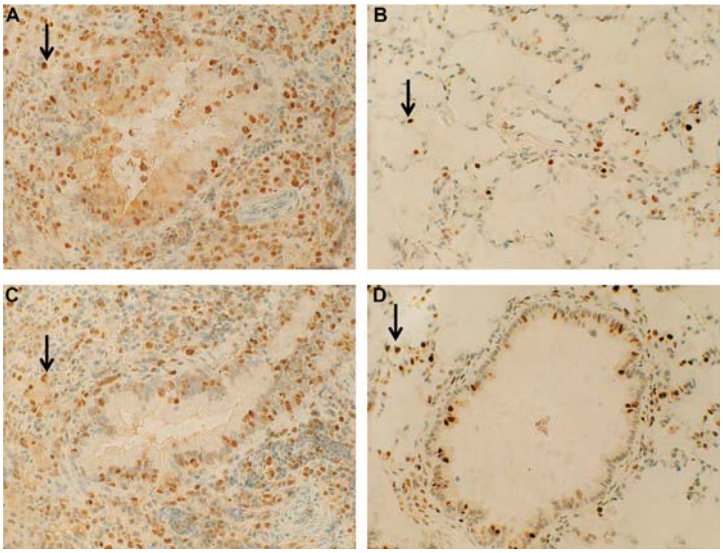
Irreversible enzymatic inhibition of DPP IV has been shown to abrogate acute pulmonary rejection, maintain pulmonary function, and preserve histomorphologic architecture. These results extend earlier findings and illustrate the role of CD26/DPP IV in alloantigen-mediated immune responses.



Structure of compound AB192 [bis(4-acetamidophenyl) 1-(S)-prolylpyrrolidine-2(R,S)-phosphonate], the irreversible DPP IV inhibitor used in this study.



Left: PO2 in inhibited (gray) vs control (black) LEW recipients of orthotopic left lung transplants from LBNF1 donors. Specific inhibition of DPP IV enzymatic activity significantly preserved oxygenation capacity of treated recipients compared with rejecting controls up to Day 5 post-Tx.
Right: Peak airway pressure (PawP) in inhibited (gray) vs control (black) LEW recipients of orthotopic left lung transplants from LBNF1 donors. Specific inhibition of DPP IV enzymatic activity preserved the significantly reduced PawP in comparison to rejecting controls up to Day 5 post-Tx.



PCNA staining of LBNF1 pulmonary allografts, transplanted into LEW recipients, on Day 5 post-Tx. Brown–red nuclei indicate PCNA-positivity (arrow). Perivascular infiltration in untreated recipients (A) was massive, with a significant increase in PCNA-positive cells, compared with treated animals (B) (controls: 55.9 ± 8.4%; inhibited: 34 ± 11.5%; $p < 0.05$). Analysis of bronchial epithelial cells in untreated (C) vs treated (D) recipients also showed a significant increase in PCNA positivity (controls: 57.2 ± 4.5%; inhibited: 31.7 ± 10.6%; $p < 0.05$) (original magnification ×200).

Functional evaluation of non-heart-beating donor lung grafts: The effect of topical cooling solution and bronchoalveolar lavage fluid nitrite/nitrate and cytokine levels as a predictor of pulmonary graft viability

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

To investigate whether the type of the topical cooling solution (saline versus low potassium dextran glucose solution (Perfadex)) has an influence on the graft preservation retrieved from NHBDs after substantial periods of warm ischemia (1, 2, and 3 hours). Second, whether bronchoalveolar lavage (BAL) nitrite and inflammatory cytokines (interleukin-1 beta (IL-1 beta), IL-6, IL-8, TNF alpha) accumulate in BAL fluid after preservation. Finally whether nitrite and these cytokine accumulation could predict the pulmonary graft viability in an ex vivo lung reperfusion model.

30 domestic pigs (30-38 kg) were randomly assigned to six groups (n=5/group).

In the heart-beating donor (HBD) group which has been used as a control group lungs were flushed with cold (4°C) low potassium dextran glucose (Perfadex) solution, explanted, and stored in the same solution (4°C) for 4 hours. In the other study groups (NHBD), pigs were sacrificed and left untouched with increasing time intervals of 1, 2 and 3 hours. Then the lungs were topically cooled with saline through intrapleural drains in group II (NHBD-S1), group III (NHBD-S2), group IV (NHBD-S3). In group V (NHBD-P1) and group VI (NHBD-P3) topical cooling was done with Perfadex. The total in situ ischemic time in all groups was 4 hours prior to assessment.

Evaluation of the Graft:

Left lungs were prepared for evaluation in ex vivo reperfusion and ventilation circuit (Fig.1). PAP and temperature of the oxygenated inflowing perfusate are gradually increased. This is called warming up during reperfusion. PAP is increased following a strict protocol. During the first 15 minutes of the reperfusion, PAP is kept between 5 and 10 mm Hg. Thereafter the pressure is increased with 1 mm Hg per minute and this to a maximum of 20 mm Hg at 25 minutes. Ventilation is started when the temperature of the effluent reached to 32°C. The oxygen flow (0.4 L/min) through the gas exchanger is switched at that moment to a mixture of CO_2 (9%) and N_2 (91%) to deoxygenate the perfusate. Tidal volume and positive end-expiratory pressure are slowly increased up to a minute ventilation of 1,9 L (approximately 40% of the in-vivo minute volume) and 5 cm H_2O, respectively, with a frequency of 20 breaths per minute (FiO_2 = 0.5). Airway pressure is continuously monitored. When PAP and the temperature of the lung reached 20 mmHg and 37.5°C, respectively, functional assessment is performed during 20 minutes of period.

Isolated reperfusion circuit (Fig.1):

The perfusion circuit comprised a bypass roller-pump, a cardiopulmonary bypass membrane oxygenator supplied with 91% nitrogen and 9% carbon dioxide gas serving as a deoxygenator, a venous reservoir, a heat exchanger and polyethylene tubing.

Before entering the pulmonary circulation, pulmonary artery flow, pulmonary artery pressure (PAP) and temperature of the perfusate are recorded online on the inflow cannula. Left atrial pressure, flow and perfusate temperature are also recorded online from the outflow cannula.

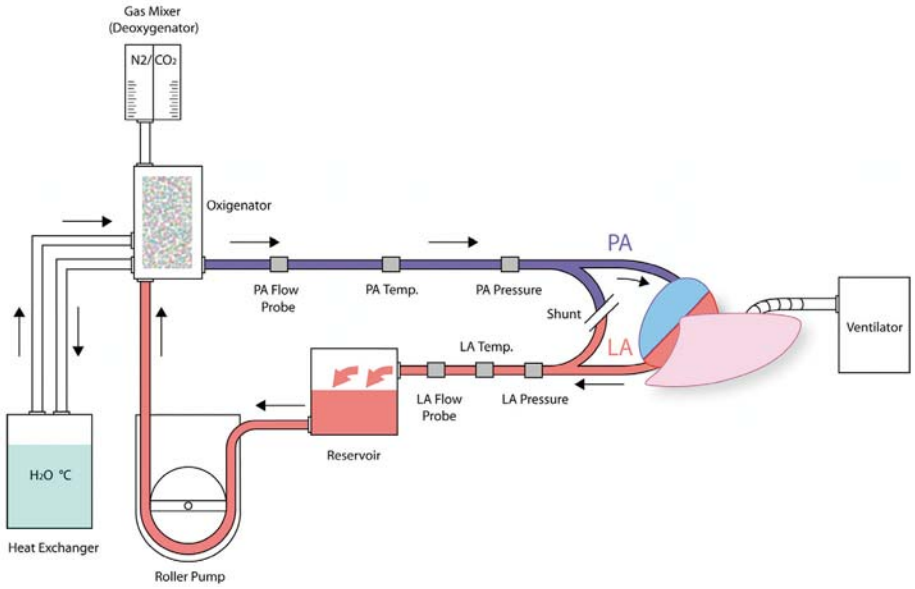


Fig. 1. Schematic diagram for the ex vivo reperfusion ventilation circuit.

Graft parameters:

Bronchoalveolar Lavage (BAL) were obtained from each right lung immediately after explantation by instillation of 60 ml saline solution using a 14 Fr catheter inserted deeply in the right main bronchus. Saline was left in place for 1 minute before aspiration. Samples were centrifugated at 3500 rpm at 4°C for 10 minutes. The supernatant was stored at -80°C until assay. The BAL nitrite level was analysed by a modified Griess reagent. The absorbances were measured at 550 nm using a microplate reader. Nitrite concentrations (micromol/liter) was calculated by comparison with optical density at 550 nm of standard solutions of sodium nitrite. BAL cytokine levels were measured by available ELISA kits. During a 20 minutes interval, three consecutive measurements of pulmonary artery (PA) flow (L/min), pulmonary artery pressure (PAP)(mmHg), left atrial pressure (LAP) (mmHg), and mean airway pressure (mmHg) were recorded and three blood gas samples were taken from the outflow perfusate to analyze oxygenation.

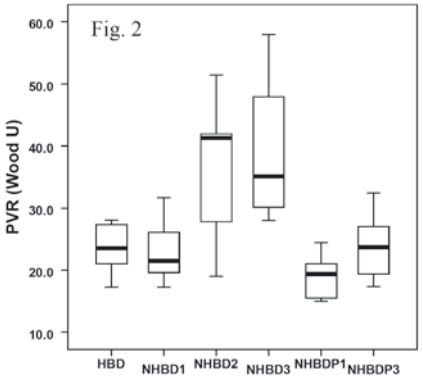
Oxygenation Index (OI) is calculated as [FiO2 (%) X mean airway pressure (mmHg) / pO2 (mmHg)] and pulmonary vascular resistance as [(PVR) = (PAP) – (LAP) / pulmonary artery flow (Wood Units)].

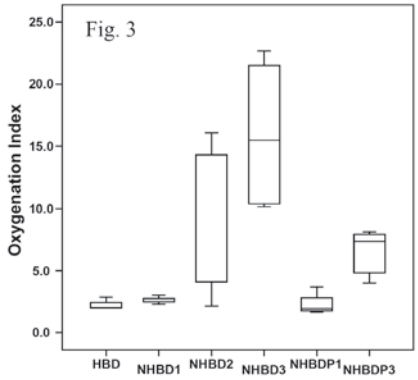
At the end of the isolated perfusion, the left lung is excised from the heart, weighed and dried in an oven at 180°C overnight. The wet-to-dry (W/D) weight ratio is calculated as an estimate of lung edema.

Pulmonary vascular resistance (PVR): PVR significantly differed among the groups (p=0.03). PVR was increased with longer warm ischemic periods. PVR was significantly higher in NHBD-S3 group compared to HBD (p=0.016), NHBD-S1 (p=0.032), NHBD-P1 (p=0.016), and NHBD-P3 (p=0.05) group **(Fig.2)**.

PVR also differed significantly between NHBD-S1 and NHBD-S3 (p=0.03).

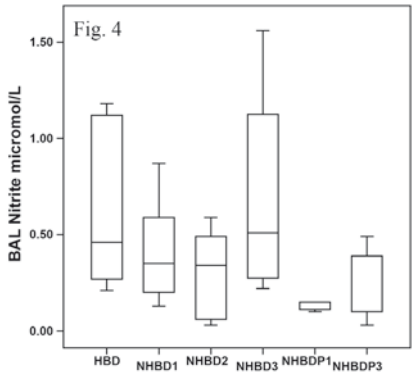
There was no statistically significance between HBD and NHBD-S1 (p=0.84), HBD and NHBD-P1 (p=0.15), HBD and NHBD-P3 (p=1), NHBD-S1 and NHBD-P1 (p=0.2), NHBD-S1 and NHBD-P3 (p=0.8), NHBD-P1 and NHBD-P3 (p=0.2).





Oxygenation Index (OI): OI significantly differed among the groups (p=0.001). OI significantly differed between HBD and NHBD-S3 (p=0.016), HBD and NHBD-P3 (p=0.008), NHBD-S1 and NHBD-S3 (p=0.016), NHBD-S1 and NHBD-P3 (p=0.008), NHBD-S3 and NHBD-P1 (p=0.016), NHBD-S3 and NHBD-P3 (p=0.016), NHBD-P1 and NHBD-P3 (p=0.008) **(Fig.3)**.

However, there was no statistical significance between HBD and NHBD-S1 (p=0.15), HBD and NHBD-P1 (p=1), NHBD-S1 and NHBD-P1 (p=0.5).



Correlations: We analyzed the correlation between BAL nitrite **(Fig.4)** and graft parameters (PVR, OI, PAWP, W/D weight ratio). There was a positive correlation between nitrite levels and PVR (r = 0.54; p=0.002). There was no correlation between nitrite and OI, PAWP, W/D weight ratio (r= 0.17, r = 0.32, r = 0.06, respectively).We also found a positive correlation between BAL protein levels and PVR (r = 0.56, p = 0.001).

Conclusions:

- A prolonged warm ischemic interval resulted in deterioration of graft function.
- Topical cooling after 1 hour of warm ischemic time is an effective method for lung preservation comparable to HBDs.
- Topical cooling with Perfadex after 3 h of death resulted in lower PVR, higher oxygenation, and lower AWP compared to saline group (NHBD-P3 vs. NHBD-S3)
- After 1 h of warm ischemia graft parameters were comparable between saline and perfadex groups
- Nitrite accumulated in BAL after death. After 1 hour of warm ischemia nitrite decreased and after 3 hours of warm ischemia it increased. Topical cooling with Perfadex resulted in lower inflammation compared to saline (NHBD-S1 vs. NHBD-P1 p=0.04)
- BAL nitrite has a positive correlation with PVR.
- BAL IL-8 tended to increase by increasing warm ischemic time. Topical cooling with Perfadex had a lower level of IL-8 after 3 h of warm ischemia compared with saline. But this was not statistically significant.
- BAL protein level increased by time and was highest after 3 hours of warm ischemic time in animals topically cooled with saline. Topical cooling with Perfadex resulted in significant reduction of BAL protein levels compared to saline. We found a positive correlation between protein and PVR and nitrite levels.

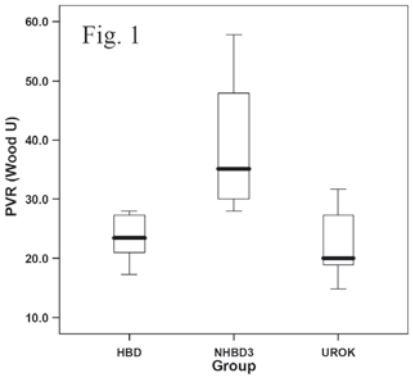
Fibrinolytic treatment improves the quality of lungs retrieved from non-heart-beating donors

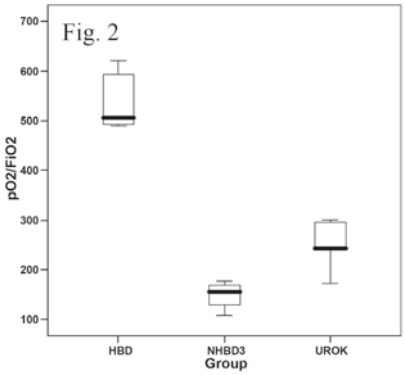
I.Inci, W. Zhai, S. Arni, S. Hillinger, B. Leskosek, W. Weder

The use of non-heart-beating donors (NHBDs) is an alternative strategy to increase the limited number of donors. The ex vivo evaluation has been proposed to assess the function of the lung from NHBDs as an interim evaluation of the graft prior to transplantation. We evaluated the effect of fibrinolytic agent, urokinase, in a pig ex vivo evaluation model.

Domestic pigs (30-38 kg) were sacrificed and divided in 3 groups (n=5/group). In the control heart-beating donor (HBD) group, the lungs were flushed, explanted, and stored in cold (4ºC) low potassium dextran (Perfadex) solution for 4 hours. In the other 2 study groups (NHBD and NHBD-UROK) the pig lungs were topically cooled for 1 hour in the closed chest after 3 hours of warm ischemia. The left lung was prepared for evaluation in an ex vivo reperfusion circuit. In NHBD-UROK group urokinase, 120.000 IU, was added into the perfusate during reperfusion. Hemodynamic, aerodynamic and oxygenation parameters were measured. Wet-to-dry weight ratio (W/D) was calculated.

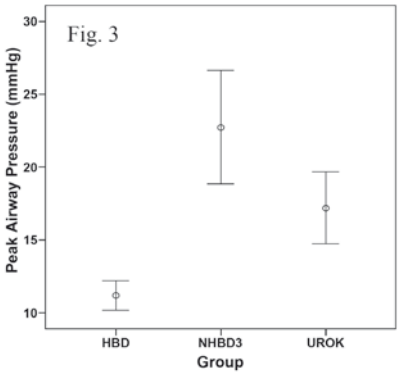
Pulmonary vascular resistance (PVR): PVR significantly differed among the groups (p=0.03). PVR was significantly different between NHBD-S3 versus Urokinase (UROK) group (p=0,032) and HBD versus NHBD-S3 group (p=0.032). PVR between HBD and UROK group was comparable (p= 0.6) **(Fig. 1)**.





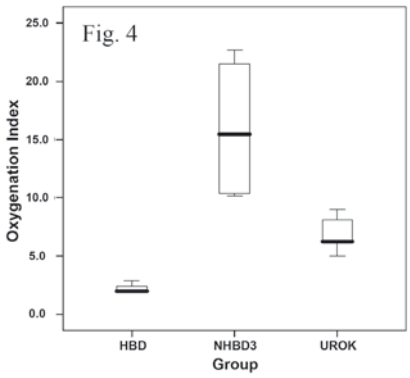
Oxygenation (PaO2/FiO2): Oxygenation significantly differed among the groups (p=0.004). Oxygenation was significantly different between HBD and NHBD-S3 (p=0.016), HBD and UROK (p=0.008), NHBD-S3 and UROK (p=0.032) **(Fig. 2)**.

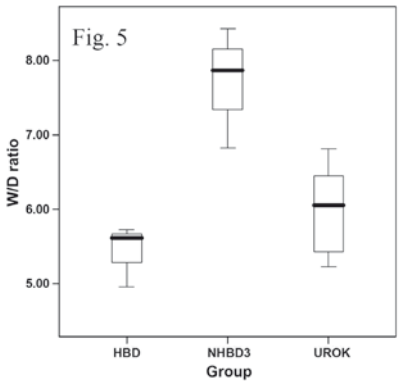
Peak airway pressure (PawP): PawP significantly differed among the groups (p=0.006). PawP was significantly different between HBD and NHBD-S3 (p=0.016), HBD and UROK (p=0.008) **(Fig. 3)**. There was no statistically significant difference between NHBD-S3 and UROK (p=0.1).



Oxygenation Index (OI): OI significantly differed among the groups (p=0.003). Oxygenation index was significantly different between HBD and NHBD-S3 (p=0.016), HBD and UROK (p=0.008), NHBD-S3 and UROK (p=0.016) **(Fig. 4)**.

Wet to dry (W/D) weight ratio: W/D weight ratio significantly differed among the groups (p=0.019). W/D weight ratio was significantly different between HBD and NHBD-S3 (p=0.05), NHBD-S3 and UROK (p=0.016). There was no statistically significant difference between HBD and UROK (p=0.3) **(Fig 5)**.





Conclusion: Adding urokinase into the perfusate during ex vivo evaluation resulted in improved graft function by reducing PVR and increasing oxygenation after 3 hours of warm ischemia. This ex vivo evaluation model is feasible and may be used to recondition grafts from NHBDs.

Achievements 2006

- Melatonin, CD26, NHBD manuscripts in preparation

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

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  J Heart Lung Transplant. 2006 Sep;25(9):1109-16. Epub 2006 Aug 17.
- Jung FJ, Cardell M, Yang L, De Meester I, Augustyns K, Hillinger S, Scharpe S, Weder W, Korom S. The role of CD26/DPP IV in preservation of early pulmonary graft function. Adv Exp Med Biol. 2006;575:231-5.
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2.5.2 Tissue Engineering


Qiang Tan


Manfred Welti

Continuous medium flow delivery chondrocytes into clinically relevant sized scaffolds - towards an in-vivo bioreactor design for tissue engineered trachea

Q.Tan, M.Welti

In our previous study on tissue engineered trachea, we put forward a novel “in-vivo bioreactor” concept defined as the design of a perfusion system inside biodegradable scaffold. Through an assistant pump system we successfully create a continuous medium flow inside the tissue engineered trachea. Our purpose is to combine the in vitro cell-scaffold construct reconstruction and in-vivo organ regeneration parts in tissue engineered researches which are traditionally separated and should be a way more physiological in organ regeneration and more practical in clinical application. In this follow-up project we tested the possibility of seeding chondrocytes by the in-vivo bioreactor design in clinic relative sizes and to assess the impact of medium perfusion on subsequent cell proliferation.

A test model of in-vivo bioreactor design was established by inserting a porous catheter connected to a pump system inside the porous scaffold. One pump continuously delivered medium into the scaffold while the other suck the waste out. The possibility of chondrocytes delivery through this intra-scaffold medium flow was tested with Poly (ethylene glycol)- terephthalate – poly (butylenes terephthalate) PEGT/PBT and Hydroxyapatite/Tricalcium phosphate HA/TCP scaffold in clinic relevant size. The impact of this perfusion approach on the proliferation of seeded cells was also compared with conventional static culture methods. Scanning electron microscope (SEM) pictures were used to evaluate the distribution of cells within the scaffolds ten days after cell seeding. All the SEM pictures proved that chondrocytes can be successfully delivered to these two large size scaffolds through the perfusion system and a better three-dimensional cell growth compared with static culture method was found in all perfusion samples. These results proved that a continuous medium perfusion inside the scaffold allowed efficient cell delivery; improved the extent of proliferation and even distribution of seeded cells inside clinic relevant sized scaffolds.

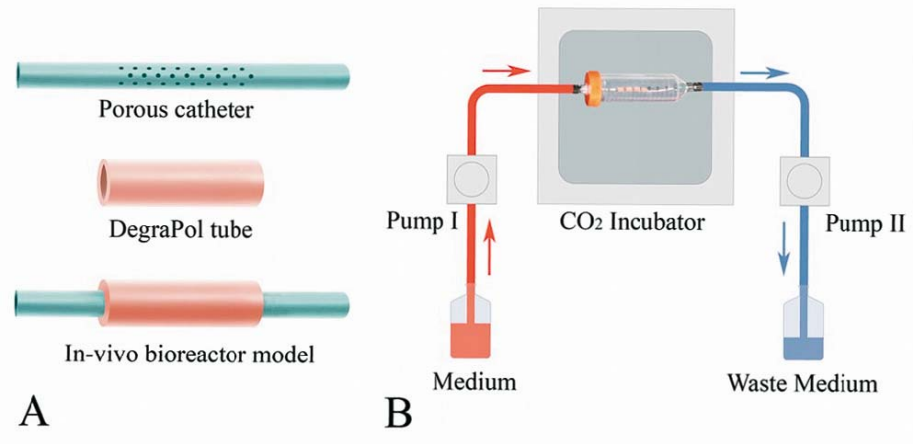


Fig.TE1. Sketch of “in-vivo bioreactor” test model

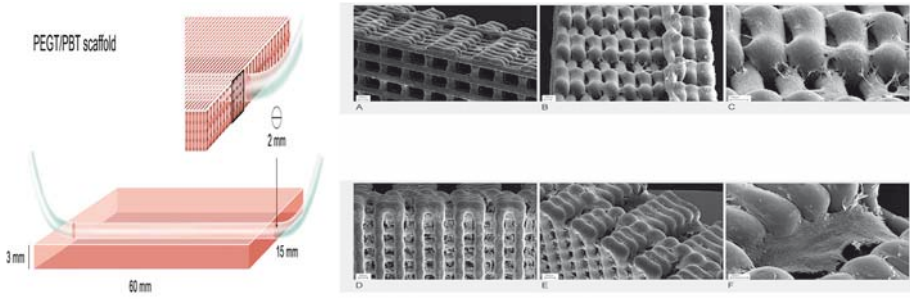


Fig. TE2. PEGT/PGT scaffold static cell seeding and tissue culture group: A. overview of the scaffold showed no three-dimensional cell growth; B. only a few chondrocytes adhered to the surface of the scaffold; C. the chondrocytes hardly formed a monolayer on the scaffold surface.
In the perfusion group: D. three- dimensional cell growth was detected inside the pores of the scaffold; E. the chondrocytes covered most of the surface of the scaffold; F. cell clusters were found trying to cover a large size hole of the scaffold.

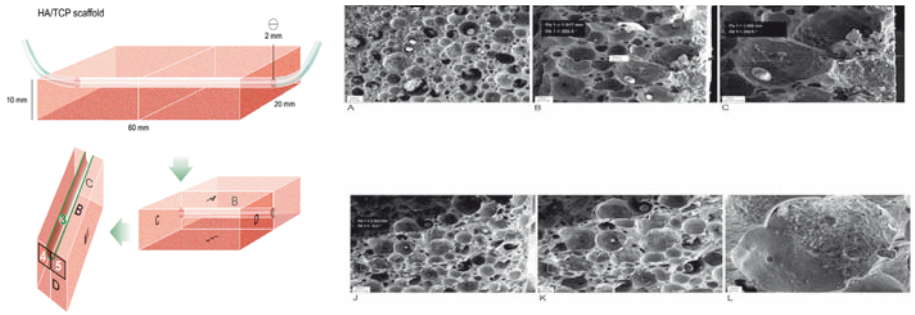


Fig. 5. HA/TCP static seeding and culture group: A. overview of the surface of HA/TCP scaffold covered with cells, exact position marked with A1 in the sketch; B. horizontal cutting surface of the scaffold showed that chondrocytes migrated into the deep part of the scaffold, exact position marked with B2 in the sketch; C. Higher magnification of picture B showed the cells only invaded less than 1mm into the HA/TCP scaffold.
Perfusion group: D. the surface of the perfusion channel was covered by chondrocytes, exact position marked 3 in the sketch; E. higher magnification showed the cells close the channel surface pores of the scaffold; F. three-dimensional cell growth was found inside the pores of the scaffold.

Achievements 2006

- Oral presentation at the Regenerate World Congress on Tissue Engineering and Regenerative Medicine, April 27, 2006 in Pittsburgh, Pennsylvania, several grant applications running

Collaborations:

- Prof. Gauckler, Institute of Nonmetallic Inorganic Materials ETHZ, Zurich, Switzerland
- Dr. L. Moroni, Twente Univeristy, IsoTis S.A., Bilthoven, Netherland

Selected references:

- Tan Q, Steiner R, Hoerstrup SP, Weder W. Tissue-engineered trachea: History, problems and the future. Eur J Cardiothorac Surg. 2006 Nov; 30(5):782-6.

2.5.3 Oncology


Dr. med.
Sven Hillinger


Dr. med.
Stephan Arni


Dr. med.
Isabelle Opitz


PD Dr. med.
Didier Lardinois


Manfred Welti


Dr. med.
Markus Cardell


Dr. med.
Brigit Oberreiter

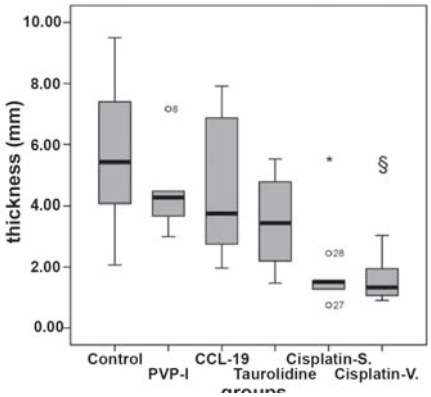
Malignant pleural mesothelioma –intrapleural therapy after surgery
I.Opitz, S.Arni, S.Hillinger, D.Lardinois

Malignant pleural mesothelioma is an aggressive tumor with increasing incidence that is expected to peak in the next two decades. The management of these patients is still controversial, with currently the best survival data after multimodality treatment including induction chemotherapy with cisplatin and pemetrexed, surgery and radiotherapy. Nevertheless, local recurrence of the tumour remains a major problem. Intrapleural therapy is an attractive treatment option for local tumor control with promising results in early clinical and experimental studies but further refinement is still necessary.

Recurrence model: A tumor cell suspension of 50 µl $1x10^6$ of rat malignant mesothelioma cells was inoculated subpleurally. 6 days later, the tumor nodule was measured and completely resected. Local recurrence at the resection site was assessed after 6 and 10 days. Local adjuvant treatment: Tumor inoculation was performed as described after labelling the cells with CFSE. After tumor resection and left sided pneumonectomy this newly developed recurrence model was used to investigate local treatment with the chemokine CCL-19, povidone-iodine, taurolidine 2%, cisplatin 100 mg^2/kg BW or cisplatin in the same concentration loaded in a fibrin-based sealant for slow release and compared with a control group. Primary endpoint was the extent of recurrence 6 days after treatment evaluated by fluorescence-microscopy. *Bioluminescent Imaging:* In a pilot project, the malignant mesothelioma cell line (II-45) was stably co-transfected with a pGL3-Control vector for expression of the luciferase gene and a pSV2 vector containing a neomycin resistance cassette. After selection, single clones were assessed for their luciferase activity. Additionally, proliferative activity of clone #9, which showed the highest luciferase activity, was compared to wild type mesothelioma. No noteworthy difference has been observed. The bioluminescent signal was evaluated by performance of in vivo imaging at day 1, 4 and 6 post inoculation after application of the substrate luciferin by positioning the animals in a chamber of the CCD camera system (Xenogen IVIS® Imaging System 100 Series). The photons emitted from the luciferase-expressing cells within the animal will be quantified for 5 min using the Living® image software. At day 6 the tumour nodule was completely resected and recurrent growth observed at day 7, 9 and 11.

Recurrence model: 6 days after tumor cell inoculation, all animals developed a tumor nodule at the injection site of a mean diameter of 5.1mm (± 0.8). At 10 days after complete resection, local and distant recurrences in the contralateral chest were found. At 6 days local recurrence only occurred.

Local adjuvant treatment: The extent of recurrence was markedly reduced by all therapy groups, whereas cisplatin-solution and cisplatin-Vivostat® significantly reduced the local recurrence in all diameters (figure 1). Histological examination revealed necrotic formation in all the tumors of cisplatin-Vivostat® or cisplatin-solution-treated animals and in 6 out of 7 animals treated with taurolidine 2%.



Bioluminescent Imaging: Bioluminescent signals were measured beginning the first day after tumor inoculation. Activity increased until the day of resection and re-increased thereafter until the time point of autopsy reflecting the growth of the tumor recurrence at the site of resection.

A local recurrence model for MPM in rats was successfully established. Herewith a significant reduction of tumour growth after local treatment with slow-released cisplatin and taurolidine was demonstrated.Non-invasive intravital tumour mapping by bioluminescent imaging was evaluated for further treatment-monitoring.

Outlook: Further refinement of chemotherapeutic intrapleural therapy and evaluation of new immunomodulatory intrapleural treatment.

Achievements 2006

Awards:

- European Young Investigator Award from the European Association of Cardio-vascular and Thoracic Surgery, Stockholm 09/06

Collaborations:

- Department of Oncology (Sally Donaldson, Rolf Stahel)
- Department of Clinical Pathology (Peter Vogt, Bernhard Odermatt)
- Institute for Biostatistics (Valentin Rousson)

Selected references:

- I. Opitz, D. Lardinois, S. Arni, S. Hillinger, P. Vogt, B. Odermatt, V. Rousson, W. Weder. Local recurrence model of malignant pleural mesothelioma for investigation of intrapleural treatment. accepted in EJCTS
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Prognostic marker for survival of malignant mesothelioma patients

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

Malignant pleural mesothelioma (MPM) is a highly aggressive tumor with poor prognosis and limited response to chemotherapy. Tumor markers may describe tumor aggressiveness and may be potential targets for therapy.
Formalin-fixed paraffin embedded tissue blocs from 470 MPM patients were retrieved from the archives of Institutes of Surgical Pathology in Switzerland and tissue micro-arrays (TMA) were constructed using quadruplicate punches. Clinical data of these patients were assessed from medical archives of the different hospitals and the local cancer registries. The expression level of the tumor supressor gene PTEN was determined by immunohistochemistry with PTEN antibody. Intensity was scored semiquantitatively 0 to 3. Statistical analysis was performed using Kaplan-Meier curves for correlation of survival time with expression of PTEN.
Clinical data from 206 patients were available and retrospectively analyzed. 104 patients were in stage T4 and 91 patients presented with regional and mediastinal lymph node metastasis. PTEN was expressed in 34% of the cases. The expression of PTEN was significantly higher in patients with epithelial mesothelioma ($p=0.0001$). The survival time was evaluated and correlated to PTEN expression in 111 cases in which follow-up data were complete. Comparing strong versus no expression of PTEN, median survival time was significantly longer (log rank test $p= 0.0001$) in patients with PTEN expression (21 months; 95% CI: 7; 36 versus 10 months; 95% CI: 8; 12).
The expression of the tumor suppressor gene PTEN seems to be strongly correlated to tumor histology and survival time in mesothelioma patients. Prospective evaluation of this marker will further elucidate its role in the context of mesothelioma treatment.
Outlook: Prospective assessment of further markers in our clinical mesothelioma database is planned. Expression profiling of cDNA arrays constructed from mesothelioma patients, analysis of the impact of development of chemotherapy resistance.

Collaborations:

- Department of Clinical Pathology (Alex Soltermann, Holger Moch)
- Institute for Biostatistics (Valentin Rousson)
- Department of Oncology (Rolf Stahel, Tom Marti)

Combined CCL19/IL-7 treatment eradicates tumors in murine models of lung cancer

S. Hillinger, S.Arni, M.Cardell, B.Oberreiter

With the existing therapeutic efforts, patients with lung cancer have a poor prognosis and less than 15% survive 5 years following diagnosis. This dismal statistic has changed minimally in the last 30 years and, therefore, new therapeutic strategies are clearly needed. One of the challenges in developing immunotherapy for cancer is enlisting the host response to recognize tumors of poor immunogenicity. It is now clear that effective antitumor responses require both antigen presenting cells (APC), lymphocyte and natural killer (NK) effectors. Although lung cancer cells express tumor antigens, the limited expression of major histocompatibility complex (MHC) antigens, defective transporter associated with antigen processing (TAP), and lack of co-stimulatory molecules make them ineffective APC. Additionally, tumor cells produce immune inhibitory factors that promote escape from immune surveillance. The tumor microenvironment can negatively affect T cell activities including those that directly abrogate effector cell function by limiting lymphocyte survival. In addition to interfering with lymphocyte responses, we as well as others have found that tumor cells inhibit dendritic cell (DC) maturation and function. Lung cancer patients have dramatically decreased numbers of circulating competent DC.

A promising way to restore tumor antigen presentation and anti-tumor effector activities is to externally enhance the level of specific chemokines and cytokines as well as functional DC at the tumor site. In preliminary results we demonstrated that systemic and locoregional treatment with recombinant EBV induced molecule 1 ligand chemokine, ELC (CCL-19), which chemoattracts DC and T cell effectors to the tumor site, in both spontaneous and orthotopic models of murine lung cancers markedly inhibit the tumor growth. Interleukin-7 (IL-7) amplifies the longevity of the tumor antigen specific T cells and NK effectors. This has also been proven by our group to be effective in reducing tumor growth in transgenic tumor bearing mice. In this study we investigated the effect of locally combined treatment with CCL19 and IL-7 on tumor growth in subcutaneous models of two different mouse strains.

105 L1C2 and 3LL cells have been injected into the right flank of Balb/C and C57/Bl6 mice respectively. Five days after injection mice have been treated intratumorally with injection of recombinant ELC/CCL19 (0.5µg/dose) three times per week and Interleukin 7 (200ng/dose) daily for 2 weeks. For the evaluation of anti-tumor responses, tumors were harvested three weeks after combined treatment and assessed for H&E staining to analyze tumor infiltrating T cell subsets by flow cytometry. Tumors were evaluated for the production of IL-10, IL-12, GM-CSF, IFNγ, TGFβ, by ELISA and PGE2 by enzyme immunoassay (EIA) in the supernatants after an overnight culture.

Local administration of CCL19/IL-7 has potent anti-tumor responses in vivo **(Fig.1)**, it enhances the frequency of T cell subsets and DCs at the tumor sites **(Fig.2)**, it promotes type 1 cytokine release as well as a decline in the immunosuppressive molecule TGFβ **(Fig.3+4)**.
Combined treatment with CCL19/IL-7 almost completely eradicates established subcutaneous tumors in two different mouse strains and leads to co-localization of both DC and T lymphocytes as well as reduction of CD4+CD25+ regulatory T cells at the tumor site. This study provides a strong rationale for further evaluation of these potent substances in the regulation of tumor immunity and their use in immunotherapy for lung cancer.

Fig. 1a

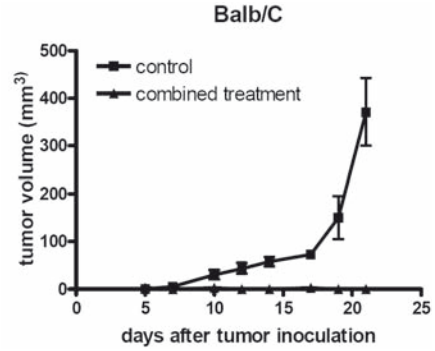


Fig. 1b

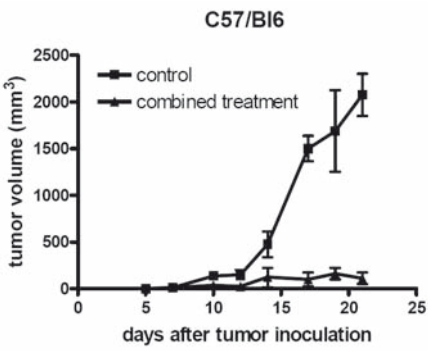
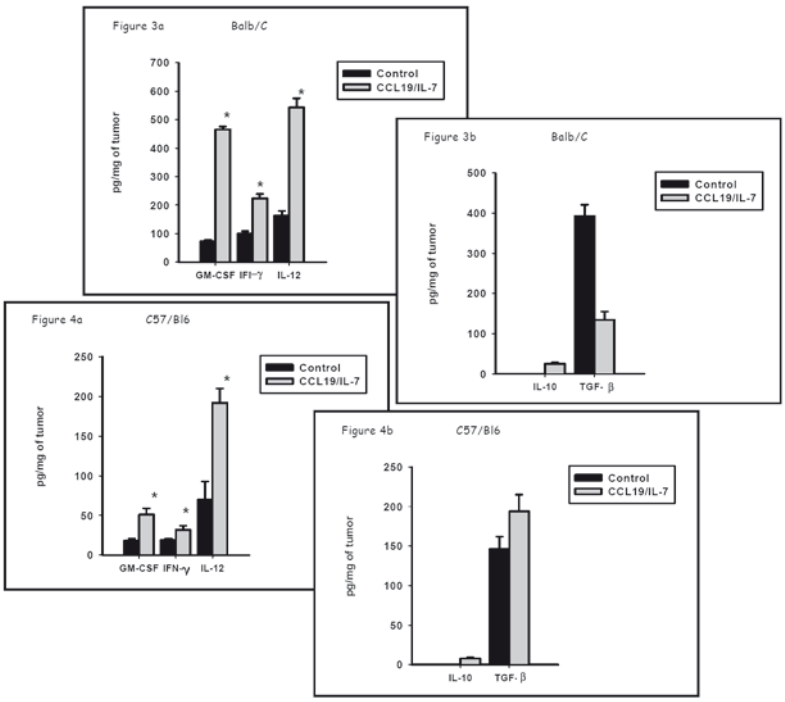


Fig.2	Control (% of lymphocytes)	CCL19/IL-7 (% of lymphocytes)
CD4	15	23*
CD8	8	16*
CD4CD25	23	11*
CD11c	9	25*



Furthermore we started to generate an adenoviral construct with genetically modified dendritic cells to express the chemokine CCL19 for enhancing the therapeutic efficacy.

Achievements 2006

- SNF-grant 'Immunotherapy for lung cancer'
- Presentations at the Annual Meeting of the AACR, Washington, DC, April 2006, ATS, San Diego, May 2006

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:

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- Hillinger S, Yang SC, Huang M, Batra RK, Strieter RM, Dubinett SM, Weder W and Sharma S. CCL19 Reduces Tumor Burden in a Murine Model of Spontaneous Bronchoalveolar Cancer. Br J Cancer Apr 94;8 (2006)
- Sharma S, Batra RK, Yang SC, Hillinger S, Zhu L, Atianzar K, Strieter RM, Riedl K, Huang M, Dubinett SM. IL-7 Gene Modified Dendritic Cells Reduce Pulmonary Tumor Burden in Spontaneous Murine Bronchoalveolar Cell Carcinoma. Hum Gene Ther. Nov;14:1511-24 (2003)

2.5.4 Clinical-related Research

The effect of NSAID application on pleurodesis – evaluation in a pig model

I.Opitz, S.Arni, B.Oberreiter, W.Zhai, D.Lardinois

It has been demonstrated that quality of adhesion obtained after pleurodesis is reduced when non-steroidal antiinflammatory drugs (NSAID) are used perioperatively. In order to elucidate the mechanisms responsible for this, we wanted to investigate the effects of NSAID administration on the early inflammatory and fibrinolytic processes during pleurodesis in an established pig model.

24 pigs were randomly assigned to either NSAID group (receiving 50 mg diclofenac peroral twice a day beginning 2 days before the intervention until autopsy) or a control group (postoperative analgesia with fentanyl patch 75 mg/h). Left sided mechanical pleurabrasion was performed by the use of videothoracoscopic assisted surgery technique. Pleural fluid (6 and 24 h) and blood samples (2, 4, 6, 8, 10, 17 and 24h) were analysed during the first 24 h following pleurabrasion. A semiquantitative histological analysis of neutrophil influx at the site of pleurabrasion was performed at 24 h by a pathologist blinded to the treatment.

We observed a significantly decreased volume of pleural secretion in the diclofenac group 10 h after pleurabrasion (t-test after log transformation). Analysis of the pleural secretion after diclofenac treatment showed a significant reduction in total protein amount ($p=0.01$), total leukocyte number ($p=0.008$), total content of TGF-beta ($p=0.007$), as well as a reduced percentage of neutrophils ($p=0.009$). Moreover, a significant increase of the fibrinolytic markers D-Dimers ($p= 0.047$) and tPA ($p= 0.025$) was observed at 6 h and of the anti-inflammatory cytokine IL-10 ($p=0.03$) at 24h. The proinflammatory cytokines IL-1b and IL-6 were reduced in the pleural fluid after diclofenac treatment but not in a significant manner. Histological analysis showed a significantly reduced number of neutrophils at the site of pleurabrasion ($p=0.007$).

Systemic application of NSAIDs led to a local enhancement of fibrinolysis and attenuation of proinflammatory and fibrotic processes necessary for adhesion formation in our pig model. These findings may explain the observed reduction of pleurodesis success after perioperative diclofenac treatment.

Collaborations:

- Department of Haematology (Lars Asmis)
- Department of Clinical Pathology (Peter Vogt, Bernhard Odermatt)
- Institute for Biostatistics (Valentin Rousson)

Selected references:

- Lardinois D, Vogt P, Yang L, Hegyi I, Baslam M, Weder W. Non-steroidal anti-inflammatory drugs decrease the quality of pleurodesis after mechanical pleural abrasion. Eur J Cardiothorac Surg. 2004 May;25(5):865-71.

2.6. Surgical Intensive Care Medicine


PD Dr. med.
John F. Stover


Prof. Dr.
Reto Stocker


Silke Ludwig


Jutta Sommerfeld

In 2006 we continued our research on platelets isolated form patients with severe traumatic brain injury (TBI) to investigate possible disadvantageous effects of norepinephrine as used in clinical routine.
Our main emphasis is to optimize our therapeutic interventions by assessing, characterizing, and excluding possible deleterious side effects related to defined therapeutic interventions as employed in our daily routine in critically ill patients. In this context, laboratory support is essental in refining clinical decision making.

Severe traumatic brain injury and secondary damage
Severe TBI is associated with locally impaired perfusion. In face of increased neuronal activity and reduced ischemic threshold the impaired perfusion contributes to progressive tissue damage. In this context, local microthrombus formation is of pathophysiological importance. Microthrombi are induced by sustained activation and adhesion of platelets to endothelial cells. This, in turn, occludes microvessels, thereby resulting in ischemia and secondary infarction. Progressive growth of cerebral edema impairs microcirculation by capillary compression. In conjunction with various destructive intra- and intercellular cascades these alterations contribute to a secondary enlargement of the primary lesion.

Platelets, adrenergic receptors and cerebral damage
Platelets are characterized by their receptor- mediated thrombogenic potential. In this setting α_2- adrenergic receptors are of pathophysiological relevance. Activation of α_2- adrenergic receptors by norepinephrine stimulates platelets and increases the risk of thrombus formation. This, in turn, can result in a sustained release of cytokines, vasoconstricting and excitotoxic mediators as e.g., IL-8, serotonin and glutamate, respectively. Upregulation of adhesion molecules promotes platelet binding and contributes to progressive impairment of posttraumatic cerebral perfusion (Figure 1). Analysis of changes in arterial and jugular venous blood could allow us to differentiate systemic from local intracerebral activation of platelets.

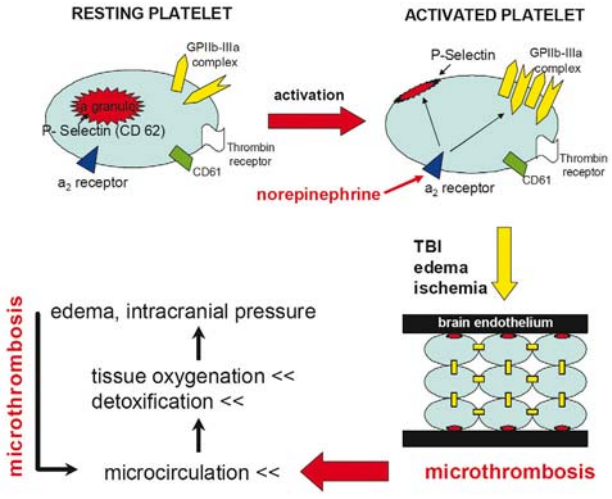

Figure 1
Schematic drawing of pathophysiologic interactions between platelets and secondary cerebral brain damage following traumatic brain injury.

Questions

1. *Does norepinephrine activate isolated platelets in patients with severe TBI under in vitro conditions?*
2. *Is there a characteristic temporal profile in activation of platelets?*
3. *Are there differences between arterial and jugular venous platelets, possibly suggesting intracerebral activation?*

Methods

For his thesis, Christoph Tschuor could evaluate a total of 11 patients. Following standardized preparation, isolated arterial and jugular venous platelets were stimulated *in vitro* with norepinephrine using different concentrations to assess a possible disadvantageous activation of platelets. Platelet activation was quantified by FACS analysis based on the expression of P- selectin and intracellular microparticles. Sustained expression of P- selectin on the surface of platelets and intracellular microparticles reflect an increased thrombogenicity of the stimulated platelets.

Results

- As previously shown in healthy controls, norepinephrine significantly activates isolated platelets in a concentration- dependent manner in vitro.
- There were significant differences between the first and second week: during the first week norepinephrine- mediated stimulation of isolated platelets was significantly suppressed in face of unchanged activation by TRAP (thrombin receptor activator protein) (Figure 2A); during the second week, however, there was an excessive norepinephrine- mediated activation (Figure 2B).

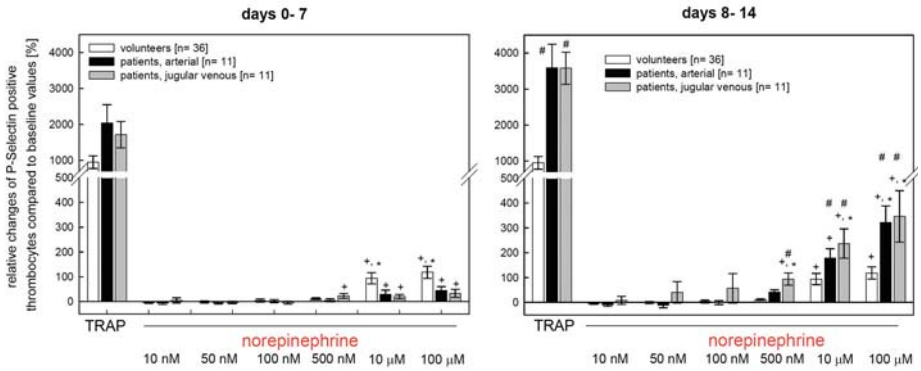

Figure 2A und 2B
Temporal profile of in vitro norepinephrine- mediated stimulation of platelets isolated from healthy controls and patients suffering from severe traumatic brain injury. While norepinephrine stimulation was significantly depressed in the first week it was significantly sustained in the second week. There was a trend to an increased stimulation of jugular venous platelets compared to arterial platelets.

- There was a trend to sustained norepinephrine- mediated stimulation of jugular venous platelets.
- However, isolated platelets which were not stimulated in vitro by norepinephrine showed normal P-Selectin expression during the first week. During the second week, P- Selectin expression on the surface of isolated and unstimulated platelets was significanty reduced, possibly reflecting state of activation associated with increased P-selectin shedding. Stimulation with TRAP was similar at all investigated time points, reflecting intact intracellular stimulation cascades (Figure 3).

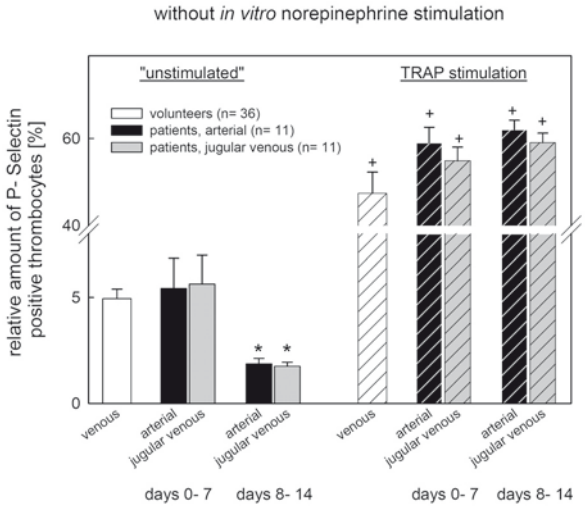

Figure 3
Differential expression of P- Selectin on platelets assessed directly following isolation without in vitro norepinephrine stimulation. In the second week there was a significant decrease in the number of P-Selectin positive cells. Interestingly, stimulation with TRAP increased the number of P-Selectin positive platelets, also in the second week, reflecting intact stimulation cascades.

Conclusions

- In vitro, norepinephrine stimulates platelets isolated from critically ill patients suffering from severe TBI in a concentration- dependent manner.
- Norepinephrine- mediated stimulation shows a characteristic functional and temporal profile.
- Reduced norepinephrine- mediated stimulation despite normal surface expression of P-Selectin during the first week could reflect functional deactivation of adrenergic receptors, maybe caused by the continuously infused norepinephrine in daily routine.
- Increased activation during the second week suggests increased receptor susceptibility to administered norepinephrine despite significantly reduced baseline expression of surface P- Selectin.

Open questions

- Do these in vitro alterations coincide with elevated norepinephrine concentrations in blood?
- Can these norepinephrine- mediated influences be blocked pharmacologically?
- Can these observed effects be avoided by using e.g., neosynephrine (phenylephrine)?
- Are there brain lesion- specific influences on isolated platelets?

Achievements 2006

- Grant approval by the Swiss National Foundation
- Employment of a lab technician in the laboratory for the Division of Surgical Intensive Care Medicine
- Integration of continuous EEG monitoring to optimize administration of sedative and analgetic agents, forming the basis for subsequent pharmacokinetic and pharmacodynamic analysis
- Establishment of high- throughput analysis of amino acids

Collaborations:

- Dr. med. Lars Asmis, Prof. Dr. Fehr, Institute for Clinical Hematology
- PD Dr. Marius Keel, Dr. rer. nat. Luc Härter, Ursula Steckholzer, Department of Surgery, Division of Trauma Surgery, University Hospital Zürich
- PD Dr. Klaus Schaser, Department of Trauma Surgery, Charité Berlin
- Prof. Dr. Unterberg, Department of Neurosurgery, Heidelberg

Selected references:

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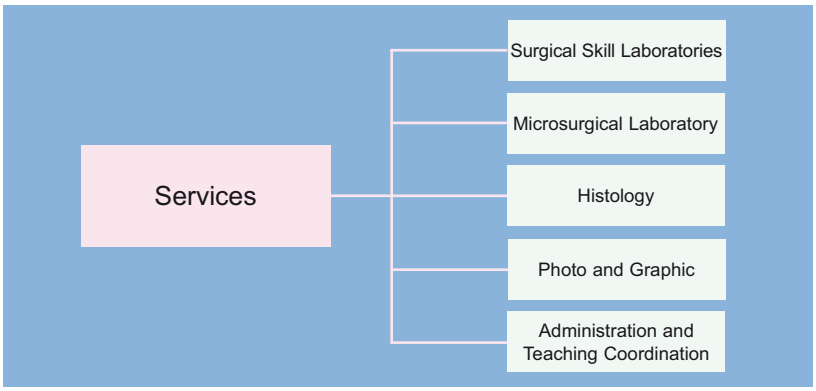
3. Services




Boris Leskosek


Alush Avdyli

3.1 Surgical Skill Laboratories

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


Vlasta Strohmeier

3.2 Microsurgical Laboratory

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


Astrid Morger

3.3 Histology

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

3.4 Photo and Graphic Services

Nico Wick,
Photographer

Lea Schütz-Cohen,
Photographer

Stefan Schwyter,
Scientific
Illustrator

Carol De Simio,
Scientific
Illustrator

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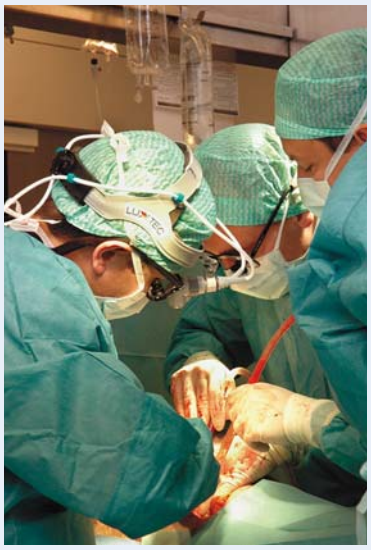
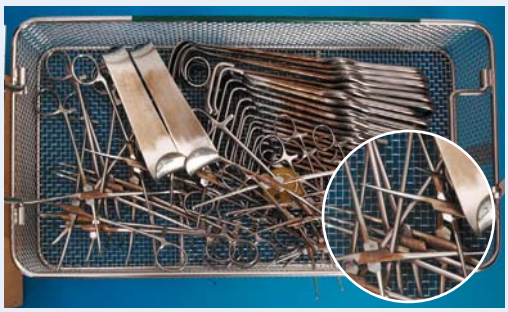
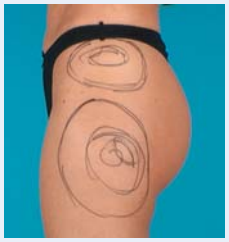
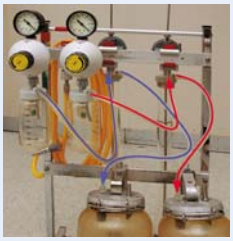
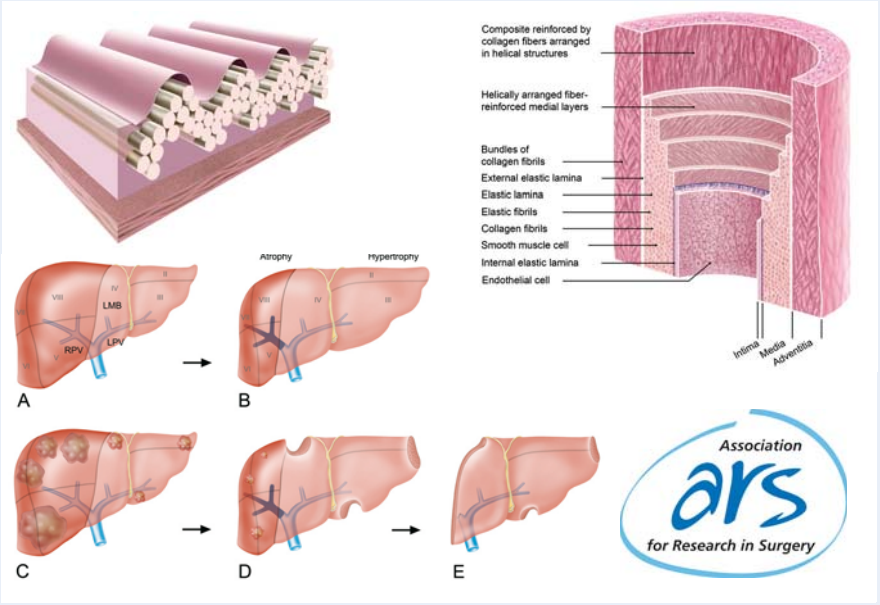
- technical photography, on location or in our well equipped studio
- reproductions from any original
- laserprint (up to A4) and inkjet (up to A3) on any material
- preparation of files for external printing
- layout of printing matters
- graphic and design of illustrations for papers and books
- construction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











Juliana Brink-Bogo,
Administration
Division of Surgical
Research

3. 5 Administration

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

Corinne Renold,
Teaching Coordination
Division of Surgical
Research

3. 6 Teaching Coordination

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

4. Events and Workshops at the Surgical Research Division in 2006

Microsurgical Laboratory

Visit of the senior civil servants of Kt. Appenzell IR

Day of clinical research

Christmas party

Sewing and injection classes for medical students

Vlasta Strohmeier's celebration of 35 years employment anniversary

5. Publications 2006

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6. Grants 2006

Cardiac Surgery

Grants	Title of Project	Project Leader
EU Grant Framework Program 6 (BioSys)	Intelligent Biomaterial Systems for Cardiovascular Tissue Repair	Prof. Zünd
Swiss National Science Foundation	Development of Cell-Based Therapies for Myocardial Defects	Prof. Hoerstrup
Hartmann-Müller-Stiftung 2006	Prenatal Human Progenitor Cells for Tissue Engineering of Living Autologous Pediatric Cardiovascular Replacements	Prof. Hoerstrup
Novartis Stiftung	Tissue Engineering Heart Valves	Prof. Zünd Prof. Hoerstrup
Bundesministerium für Bildung und Forschung (BMBF Grant)	Cryopreserved Umbilical Cord Cells For Heart Valves Tissue Engineering	Prof. Hoerstrup
Zurich Center for Integrative Human Physiology Grant 2006 – 2008	Vulnerable atherosclerotic plaques - early detection, functional imaging, targeted treatment	Prof. Hoerstrup
National Competence Center of Research	Klinik für Herz- und Gefässchirurgie - Lead Cardiac Robotic Surgery Switzerland	Prof. Zünd
SNF (NF46)	Cell and matrix evaluation in tissue engineering	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	Small-for-size liver transplantation: platelets and platelet-derived serotonin in the ischemic and regenerating liver	Prof. P.A. Clavien
NIH	Mechanismus of endothelial cell death in the ischemic liver	Prof. P.A. Clavien
Bonizzi-Theler Stitung	Die Schutzmechanismen in der ischämisch präkonditionierten Leber	Prof. P.A. Clavien
Norvartis Stiftung für Medizinisch-Biologische Forschung	Hypothermic oxygenated perfusion extracorporeal of the rat liver in non heart beating donors after cold storage	PD Dr. Ph. Dutkowski
Edoardo R., Giovanni, Giuseppe und Chiarina Sassella-Stiftung	Ceramidbasierte Behandlung des kolorektalen Karzinoms	Dr. F. Dahm/ Prof. P.A. Clavien
Hartmann Müller - Stiftung für med.Forschung	Der Einfluss von Cholestase auf Ischämie-Reperfusionsschaden in der Leber	Dr. P. Georgiev/ Prof. P.A. Clavien
Sophienstiftung	Mechanism of human liver regeneration after major hepatectomy and portal vein ligation using gene microarray technology	PD. Dr. H. Petrowsky/ Prof. P.A. Clavien
Olga Mayenfisch Stiftung	Einfluss der Thrombozyten und des darin gespeicherten Serotonins auf den normothermen Ischämie- und Reperfusionsschaden der Leber	Dr. A. Nocito Prof. P.A. Clavien
Pancreatitis laboratory		
SNF Velux Stiftung	The role of COX-2 chronic pancreatic inflammation and fibrosis The role of macrophages in chronic pancreatic inflammation	PD Dr. Graf PD Dr. R. Graf
Islet-Transplantation laboratory		
Olga Mayenfisch	Präkonditionierung	Dr. W. 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
AO Research Foundation	Assessment of soft tissue and periosteal microcirculation in severely open fractures using orthogonal polarozation spectral imaging	Dr. Wanner
Stiftung für wissenschaftl. Forschung der Universität Zürich	Nichterythroide Wirkungen von humanem rekombinaten Erythro-poietin in der Traumatologie und rekonstruktiven Chirurgie der Extremitäten	Dr. Wanner
Novartis.AO. Research Commission Hartmann Müller-Stiftung Sanofi Synthelabo	Modulation of Host Responses to Bacterial Endotoxin	PD Dr. Heinzelmann

Plastic Hand & Reconstructive Surgery

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

Thoracic Surgery

Grants	Title of Project	Project Leader
Krebsliga	Pharmacokinetic Study after Intrapleural Topical Application of Chemotherapeutic Agent for malignant Pleuramesothelioma in Immune Competent Rat Model	PD Dr. Lardinois
Hartmann Müller-Stiftung	Pharmacokinetic Study after Intrapleural Topical Application of Chemotherapeutic Agent for malignant Pleuramesothelioma in Immune Competent Rat Model	PD Dr. Lardinois
Hartmann-Müller-Stiftung	Sildenafil verlängert das Graft Überleben in einem Grosstier-Transplantations Modell	Prof. W. Weder
Olga Mayenfisch-Stiftung	Tissue-engineering zur Trachealrekonstruktion	Dr. Hillinger
Krebsliga Zürich	Adjuvante intrapleurale Spüllösung nach Pleuropneumonektomie beim malignen Pleuramesotheliom	Dr. Schmitt-Opitz
Krebsliga	Epstein Barr virus-induced molecule 1 ligand chemokine in lung cancer therapy	Dr. Hillinger
Sassella-Stiftung	Epstein Barr virus-induced molecule 1 ligand chemokine (ELC/CCL19)	Dr. Hillinger
Olga Mayenfisch-Stiftung	Assessment of the degree of pleurodesis after pleural mechanical abrasion and administration of COX-2 selective inhibitors and nitric oxide - releasing NSAI-drugs in comparison to classical NSAIDs in a pig model	PD Dr. Lardinois
Hartmann Müller-Stiftung	Immunsuppressive und zytostatische Wirkung in tumorinokkulier-ten Empfängern perfundierter Organtransplanatate	Dr. Hillinger
SNF	Trachea reconstruction using novel tissue engineered constructs	Prof. W. Weder
SNF	Immune targeted therapy for lung cancer	Dr. Hillinger
EMDO-Stiftung	Entwicklung eines in-vivo-Bioreaktors zur Reepithelialisierung einer tissue-engineerten Neo-Trachea	Dr. Hillinger
Hartmann-Müller-Stiftung	The effect of NSAIDs on early inflammatory response after mechanical pleurodesis in a pig model.	Dr. Schmitt-Opitz

Surgical Intensive Care Medicine

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

7. Awards 2006

- International Society for Applied Cardiovascular Biology, IACB-Young Investigator Award 2006, La Jolla, CA, USA, Schmidt D
- European Society of Artificial Organs (ESAO) Young Investigator Award 2006, Kelm J
- 5th Day of Clinical Research, Zurich, Switzerland: Best Poster Presentation 2006, Schmidt D
- International Academic Scholarship Award 2006, Faculty of Health Sciences, University of Cape Town, South Africa, Schmidt C
- Best Oral Presentation, Dörthe Schmidt, 4th Clinical Day of Research, Center for Clinical Research, University Hospital Zürich
- PD Dr. Tian received a poster prize from the ZKF (4th Day of Clinical Research)
- Dr. M. Lesurtel received the SGC award for his lecture at the annual meeting of the Swiss Society of Surgery.
- European Young Investigator Award from the European Association of Cardio-vascular and Thoracic Surgery, Stockholm 09/06, I. Schmitt-Opitz.

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