## Division of Surgical Research

# Annual Report 2007

## Department of Surgery
## University Hospital Zurich
## Switzerland


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

# *Content*

# *Preface*

Dear Colleagues

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

It is my privilege to present the Annual Report 2007 of the Division of Surgical Research at the Department of Surgery, University Hospital Zurich.

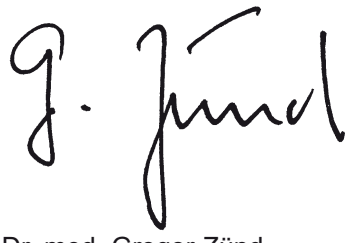
In the year 2007, two new surgical clinics, the Clinic for Urology and the Clinic for Maxillary Surgery joined the Department of Surgery. The Division of Surgical Research within the Department of Surgery was therefore enhanced by two new and active research groups, the research group for urology and the research group for maxillary surgery. These two groups will bring new inputs and projects and we are looking forward to their active integration into the Division. For both research groups, new facilities had to be built, and the Division of Surgical had to be reorganised. I would like to thank the staff of the Division for their flexibility when we were moving and the new group leaders for their cooperation and patience during reconstruction and reorganisation.

Among significant acquirements of laboratory equipment performed in 2007 were the acquisition of a fluorescence microscope, a cryomicrotome, a laboratory freezer (temperature range - 50 to -86°C), and a second-hand ultrasound system (Echosystem Sonos 5500 / Philips).

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

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

Yours sincerely



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

# 1. Organisation



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

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

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

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

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

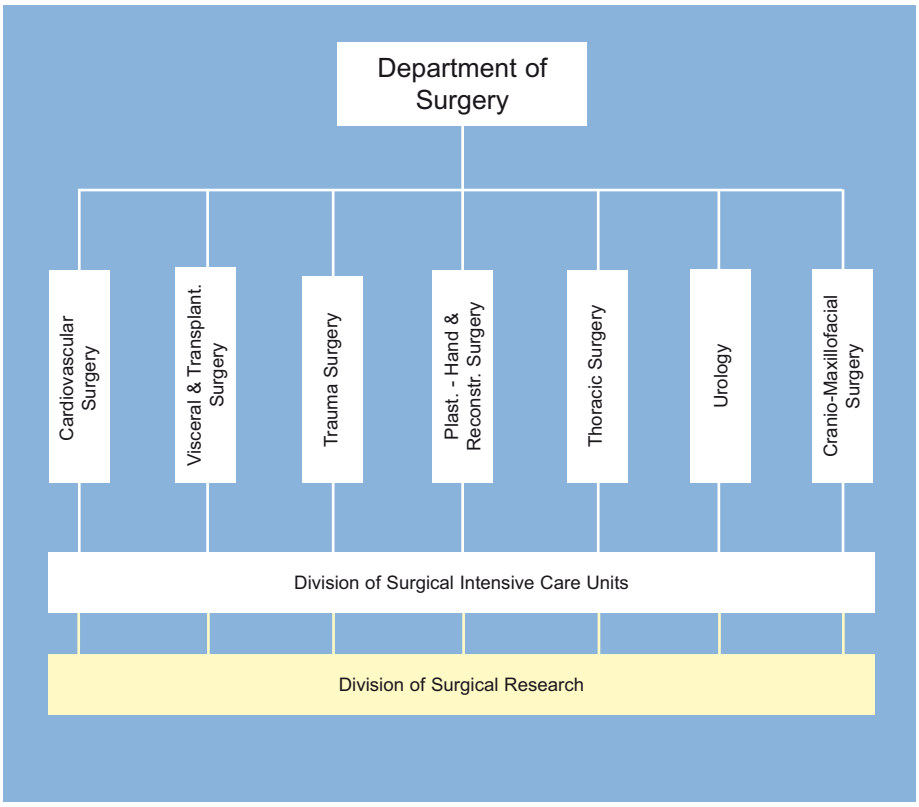
Prof. Dr. med. Pietro Giovanoli, Director Clinic of Plastic - Hand & Reconstr. Surgery

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

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

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

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

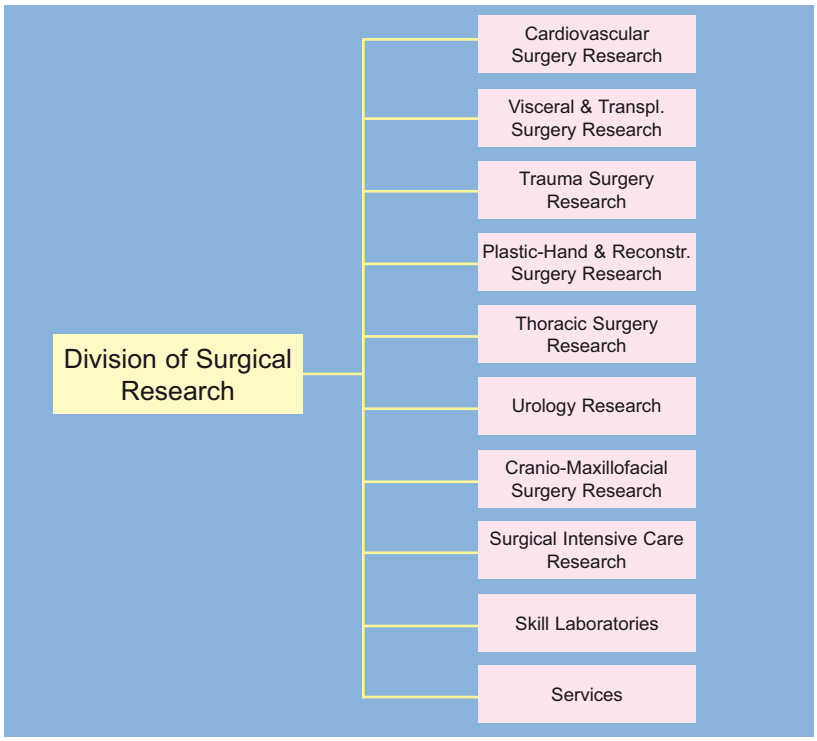


## 1.2 Structural Organisation of the Division of Surgical Research

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

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

Juliana Brink-Bogo,
Administration
Division of Surgical
Research



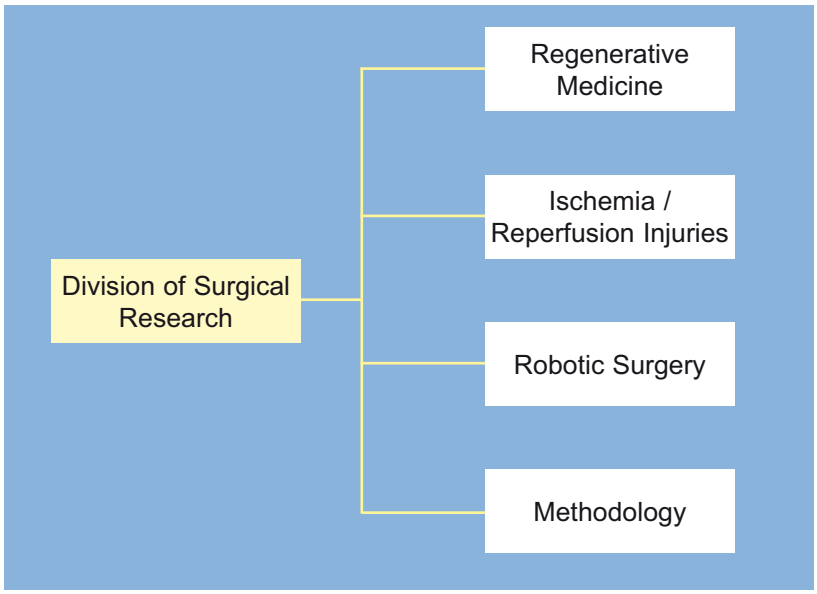

## 1.3 Scientific Sections within the Division of Surgical Research

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



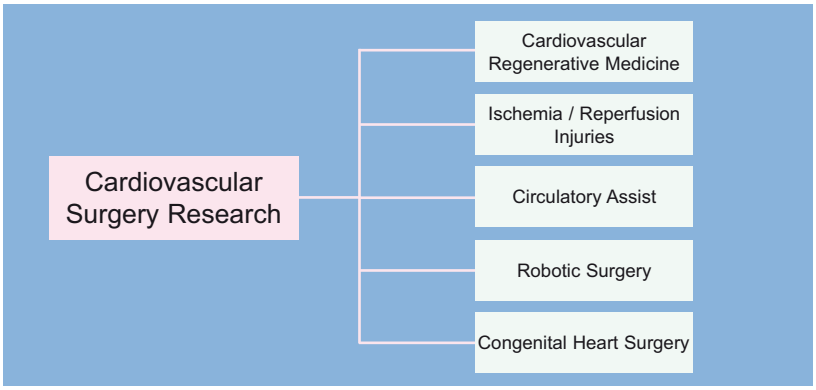
## 2.1 Cardiovascular Surgery Research


Prof. Dr. med.
Simon Philipp
Hoerstrup


Prof. Dr. med.
Gregor Zünd


Prof. Dr. med.
Michele Genoni


Sandra Edwin
Study Coordination
and Administration





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


Dr. med.
Dörthe Schmidt


Dr. sc. nat.
Jens Kelm


Dr. sc. nat.
Irina Agarkova


Dr. sc. nat.
Ronan Schönauer


Dr. med.
Christian Schmidt


Rene Stenger
Bachelor
Chemistry


Anita Mol
PhD


Dr. med.
Alberto Weber


cand. med.
Armin Zürcher


cand. med.
Silvan Holdener


cand. med.
Sandro Imbach


cand. med.
Marek Balikowski


Praktikant
Volker Lorber


cand. med.
Boris Jenni

### 2.1.1 Cardiovascular Regenerative Medicine (Tissue Engineering and Cell Transplantation)

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

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

**Research projects:**

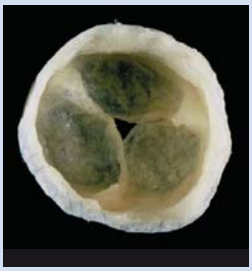
- Human Cell-Based Systems (progenitor, fetal, adult)
- Extracellular Matrix (proteins, tensegrity)
- Biomaterials (biodegradable, intelligent material systems)
- Bioreactor Systems
- Biomechanics, Computational Models, Molecular Imaging
- Animal Models (small and large)
- Tissue Engineered Cardiovascular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

cand. med.
Chad Brokopp

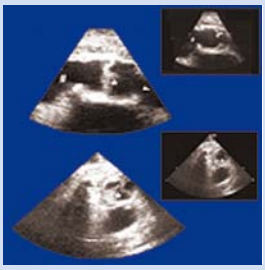
cand. med.
Pascal Heye

cand. med.
Karim Saba

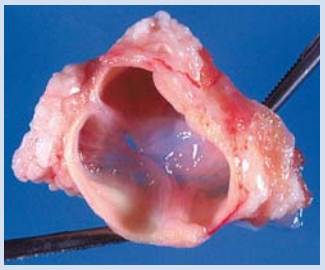
## Proof of Heart Valve Tissue Engineering Concept



pre-implantation



in-vivo

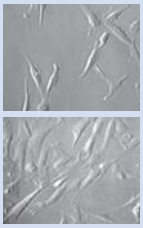


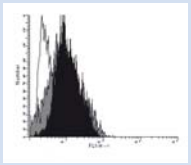
post-explantation

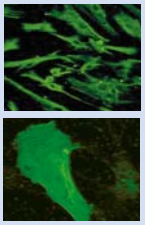
Autologous living tissue engineered heart valve in a sheep model, based on vascular - derived myofibroblasts and endothelial cells

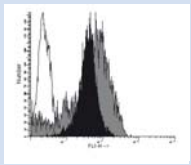
*Hoerstrup et al. Circulation 2000*

## Human Heart Valve Tissue Engineering





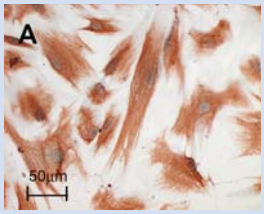


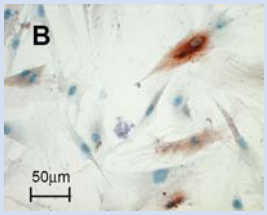


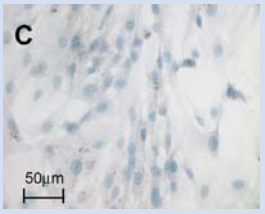
Heart valve tissue engineered from human marrow stromal cells

*Hoerstrup et al. Circulation 2002*

## Human Prenatal Stem Cells for Pediatric Cardiovascular Tissue Engineering

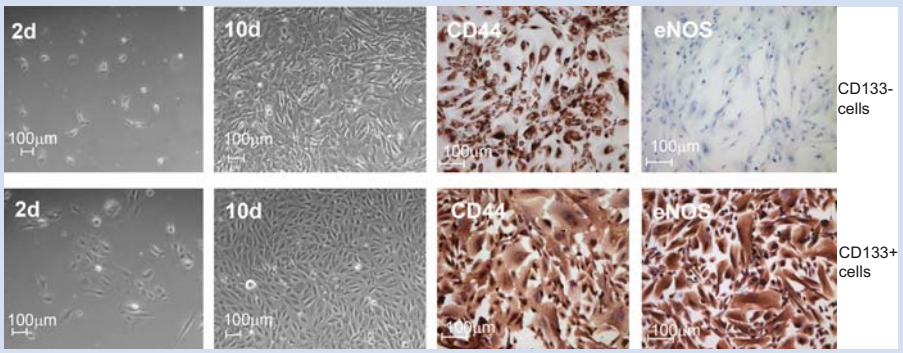



Differentiated human chorionic villi-derived prenatal progenitor cells demonstrated phenotypes similar to interstitial cells of native heart valves by expressing vimentin (A) and partly α-SMA (B) and a lack of desmin (C) and could be successfully used for the fabrication of autologous heart valves.

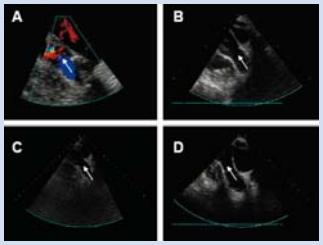
Heart valve tissue engineered from human prenatal stem cells.
*Schmidt D et al. Circulation 2006*



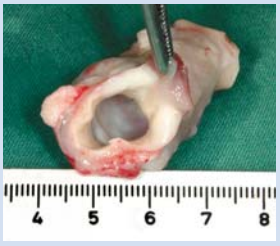

Fetal amniotic fluid-derived CD133+ and CD133- cells for autologous peditaric heart valve tissue engineering.
(*Schmidt D et al. Circulation 2007*)

## Functional Growth in Living Cardiovascular Grafts

pre-implantation




in-vivo

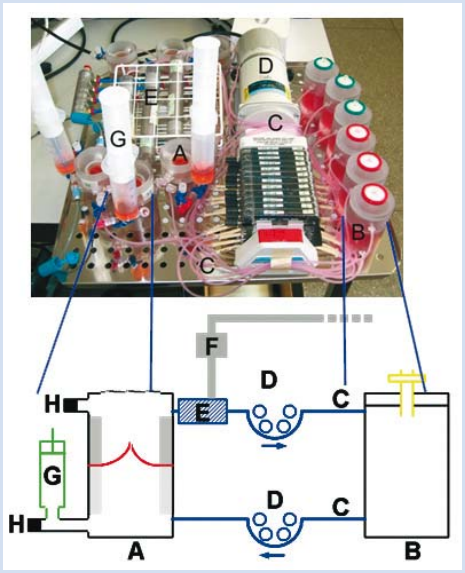



post-explantation

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

*Hoerstrup SP et al. Circulation 2006*

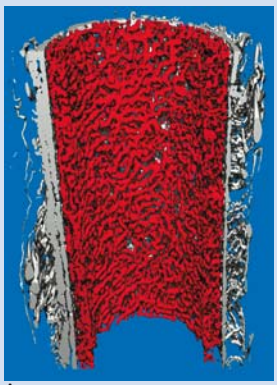
## Bioreactor Development



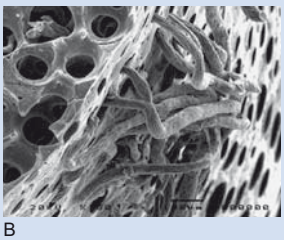

- sized small
- biocompatible materials
- maintenance of sterility
- static conditioning by continuous perfusion (4ml/min)
- dynamic conditioning by mimicking diastolic phase

*Mol et al. Ann Biomed Eng 2005*

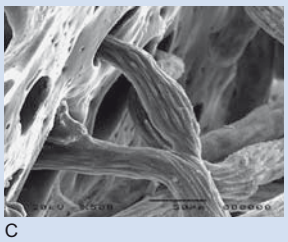
## Neovascularisation of Biomaterials through Growth Factor Delivery



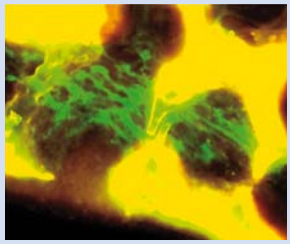
A



B



C

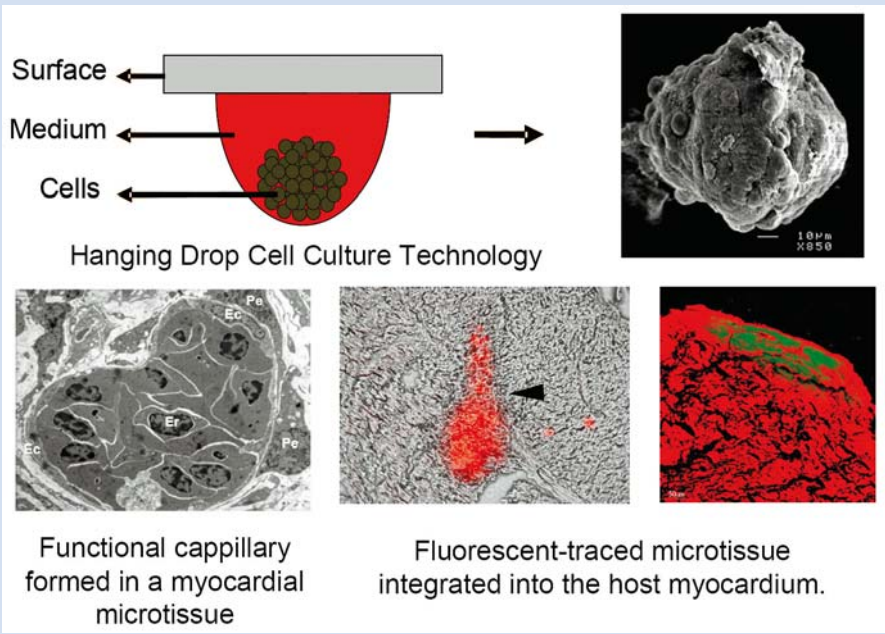


D

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

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

## Design of Microtissues for Myocardial Regeneration



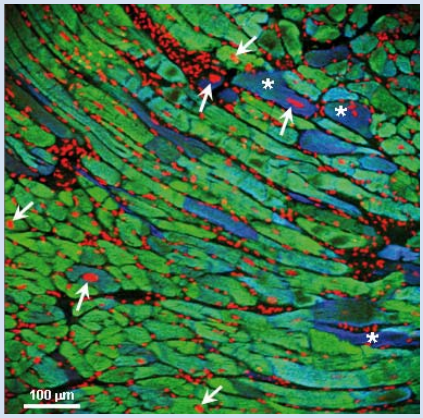

Myocardial microtissues are generated by gravity-enforced assembly of monodispersed cardiomyocytes in hanging drops. Myocardial microtissues (i) retain heart-muscle-specific morphology, (ii) express extracellular matrix components (iii) induce neovascularization, and (iv) integrate seamlessly into the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

## Characterisation of sarcomere alterations by cardiomyopathies

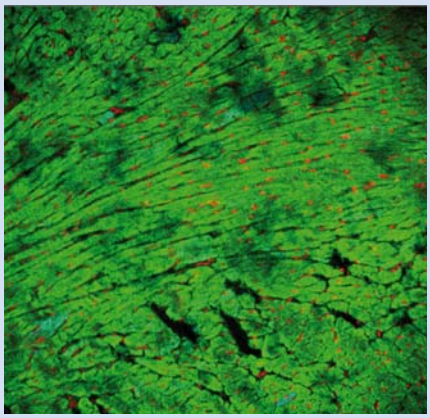
Immuno-fluorescent staining of the heart of b-catenin mutant mouse, which develops dilated cardiomyopathy and heart failure.

**Red: Nuclei**
**Green: M-protein**
**Blue: EH-myomesin**

**ß-catenin mutant heart**




**control mouse heart**



*The diseased heart is characterised by cardiomyocytes size irregularities and higher heterogeneity of the cytoskeletal components. Some cells change the M-band protein expression to a completely embryonic phenotype (*, blue staining). Numerous small nuclei outside of the cardiomyocytes area denote accumulation of fibroblasts (fibrosis) in the diseased heart. Some cardiomyocytes show hypertrophied nuclei (arrows).*

## Achievements 2007

- 2007 Kelm J.M., G. Fischer, C. Zuppinger, and S.P. Hoerstrup "Microtissue Integration into a Chronically Infarcted Myocardial Wall". 20th Meeting of the European Society for Animal Cell Technology, Dresden, Germany Winning Poster of the Roche Poster Prize
- Research grant: "M-band alterations characterize muscle pathogenesis" Swiss Foundation for Research on Muscle Diseases 2006-2008.
- Research grant: "The role of M-band in the striated muscle sarcomere" by Wolfermann-Nägeli-Foundation at 2007. Dr. Agarkova 2007-2008.
- Schmidt Christian, 2007, Zürich: PhD Cardiothoracic Surgery, University Cape Town, Medical Faculty, South Africa.
- Schmidt Dörthe, 2007, Zürich: PhD, Faculty of Biomedical Engineering, Technical University Eindhoven, The Netherlands.

## Collaborations

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

**Selected references:**


- Schmidt D., A. Mol, J.M. Kelm, and S. P. Hoerstrup (2007) „ In Vitro Heart Valve Tissue Engineering" Meth. Mol. Biol. Tissue Engineering 2nd Edition 140, 319-330.
- Sonia Meysen, Laurine Marger, Kenneth W Hewett, Thérèse Jarry-Guichard, Irina Agarkova, Jean-Claude Perriard, Seigo Izumo, Robert G Gourdie, Matteo E, Mangoni, Joël Nargeot, Daniel Gros and Lucile Miquerol. 2007. "Nkx2.5 cell-autonomous gene function is required for the postnatal formation of the peripheral ventricular conduction system." Developmental Biology. 303 (2): 740-753
- Schmidt D, Achermann J, Odermatt B, Breymann C, Mol A, Genoni M, Zund G, Hoerstrup SP. Prenatally fabricated autologous living heart valves based on amniotic fluid-derived progenitor cells as single cell source. Circulation 2007.116:I64-I70.
- Kelm J.M., W. Moritz, D. Schmidt, S.P. Hoerstrup and M. Fussenegger. (2007) "In vitro vascularization of human connective microtissue". Meth. Mol. Biol. Tissue Engineering 2nd Edition 140, 153-167.
- 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
- 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.

- 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
- 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

## 2.1.2 Ischemia / Reperfusion Injury

PD Dr. med.
Reza Tavakoli

Dr. rer. nat.
Anna Bogdanova

Dr. Deyan Mihov

**Mechanism of cardioprotection induced by Erythropoietin**
PD Dr. med. R. Tavakoli, Dr. sc. nat. Inna Agarkova,
Dr. sc. ETH Roman Schönauer, Dr. rer.nat. A. Bogdanova, Dr. D. Mihov

In our model of heterotopic heart transplantation in the rat, we have already shown that Erythropoietin significantly reduces the myocardial injury caused by ischemia and reperfusion. However, in contrast to the reports of other authors finding a significant role of apoptosis in myocardial injury following ischemia and reperfusion we could not demonstrate any sign of apoptosis in our model.
Further, in order to cast some light on the potential mechanism of cardioprotection offered by Erythropoietin we investigated the pharmacokineticks of human recombinant Erythropoietin (hr Epo). In non-treated control recipient animals of ischemic and reperfused heart grafts the native endogenous Erythropoietin plasma levels deacreased early after the onset of reperfusion and remained low during the first 6 hours thereafter (Figure 1). In hr Epo-treated recipient animals of ischemic and reperfused heart grafts hr Epo plasma levels were maximal at 5 min. after the onset of reperfusion (130±7 U/ml) and decreased over time to 3.92±0.20 U/ml at 24 hrs. of reperfusion. Using the exponential curve fitting algorithm, a half-life of approximately 146 min. was calculated for hr Epo in rat plasma (Figure 2).

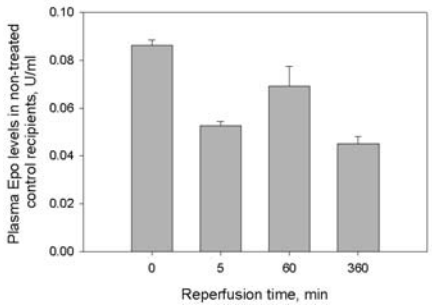



Figure 1

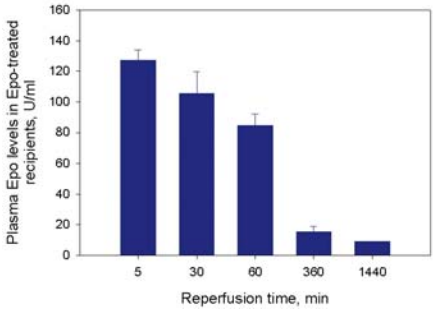



Figure 2

In hr Epo-treated recipient animals tissue levels of Erythropoietin in the ischemic and reperfused heart grafts was 100 times higher (26.3±5 mU/mg) than in the native hearts of the same recipient animals (0.26±0.11 mU/mg). Interestingly, tissues levels of ischemic and reperfused heart grafts in non-treated recipient animals was as low as in the native hearts of the recipient animals (Figure 3).

Immunohistochemical analysis of localization of Epo binding in the ischemic and reperfused heart grafts revealed that almost all Epo staining is localized within the blood vessel walls (Figure 4). This finding suggests that intravenously administered hr Epo does not reach the myocardium but adheres to the activated endothelium of the ischemic and reperfused heart grafts. Hence, we postulate that the mechanism of cardioprotection offered by Epo involves a second “endothelial” messenger. Future plan: one of the potential second “endothelial” messengers is nitric oxide (NO). We plan to investigate the effect of Epo on NO synthase activity in coronary endothelium as well as in myocardioal tissue.

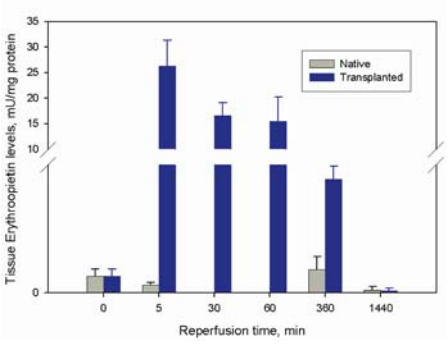



Figure 3

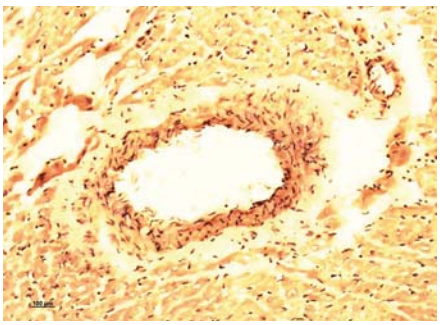


Figure 4

## Achievements 2007

- Mihov D, Grenacher B, Gassmann M, Tavakoli R, Bogdanova A. „Erythropoietin in protection of the heart against cold global ischemia-reperfusion injury“. Annual meeting of Cardiovascular Research and Training Network, Bern. February 2007
- Tavakoli R, Bogdanova A, Vogel J. SNF Grant: Ischemia and reperfusion injury in diseased heart. March 2007
- Bogdanova A, Kouroedov A, Tavakoli R, Vogel J. ZIHP Grant: Erythropoietin: protecting the heart from cold global ischemia-reperfusion injury. September 2007
- Bogdanova A., Tavakoli R. “Erythropoietin-induced cardioprotection”.Invited lecture at the Seminar of the Institute of Molecular and Cellular Biology, Medical Department, University of Saarland, Homburg, Germany. November 2007

## Collaborations:

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

## Selected references:


- Wright, G.L. et al. Erythropoietin receptor expression in adult rat cardiomyocytes is associated with an acute cardioprotective effect for recombinant erythropoietin during ischemia-reperfusion injury. Faseb J 18, 1031-3 (2004).
- Parsa, C.J. et al. Cardioprotective effects of erythropoietin in the reperfused ischemic heart: a potential role for cardiac fibroblasts. J Biol Chem 279, 20655-62 (2004).
- Hausenloy, D.J. & Yellon, D.M. New directions for protecting the heart against ischaemia-reperfusion injury: targeting the Reperfusion Injury Salvage Kinase (RISK)-pathway. Cardiovasc Res 61, 448-60 (2004).
- Ananthakrishnan, R., Hallam, K., Li, Q. & Ramasamy, R. JAK-STAT pathway in cardiac ischemic stress. Vascul Pharmacol 43, 353-6 (2005).
- Chalecka-Franaszek, E. & Chuang, D.M. Lithium activates the serine/threonine kinase Akt-1 and suppresses glutamate-induced inhibition of Akt-1 activity in neurons. Proc.Natl.Acad.Sci.U.S.A 20;96, 8745-8750 (1999).
- Jope, R.S. Lithium and GSK-3: one inhibitor, two inhibitory actions, multiple outcomes. Trends Pharmacol Sci 24, 441-3 (2003).
- Tramontano, A.F. et al. Erythropoietin protects cardiac myocytes from hypoxia-induced apoptosis through an Akt-dependent pathway. Biochem Biophys Res Commun 308, 990-4 (2003).
- Ganter MT, Monn A, Tavakoli R, Genoni M, Klaghofer R, Furrer L, Honegger H, Hofer CK. Monitoring activated clotting time for combined heparin and aprotinin application: in vivo evaluation of a new aprotinin-insensitive test using Sonoclot. European journal of cardio-thoracic surgery 30, 2 278-84 (2006).
- Reuthebuch O, Weber A, Hofer C, Burkhard R, Tavakoli R, Genoni M. Koronarrevaskularisation ohne Herz-Lungen-Maschine bei Thrombozytopenischer Purpura (M. Werlhof) Kardiovaskuläre Medizin 9, 78-80 (2006).
- Tavakoli R, Gazdhar A, Pierog J, Bogdanova A, Gugger M, Pringle IA, Gill DR, Hyde SC, Genoni M, Schmid RA. Electroporation-mediated interleukin-10 overexpression in skeletal muscle reduces acute rejection in rat cardiac allografts. The journal of gene medicine 8(2) 242-8 (2006).

## 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 2007, 13 patients were supported with this device (fig. 1). Eight patients were transplanted successfully, and one patient was switched to a biventricular device . Seven 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. Until end of 2007,13 patients were supported with the EXCOR. Nine patients were discharged home with the device during the waiting time for heart transplantation. Seven patients were transplanted, one could be weaned, two died, and three are currently still on support.

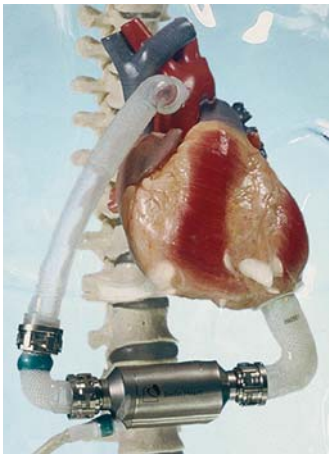


Figure 1 Berlin Heart INCOR

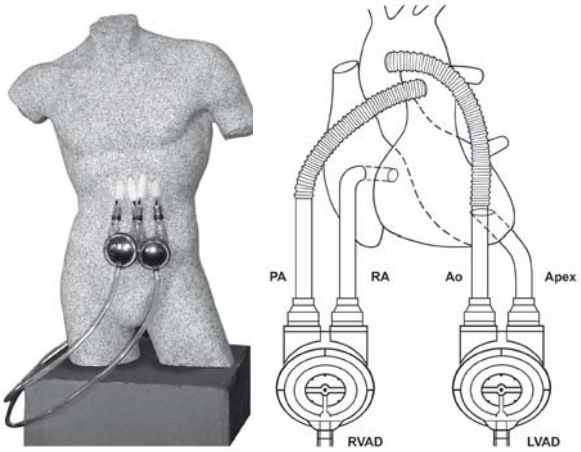
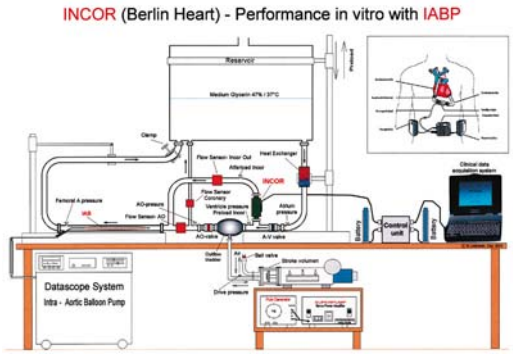



Figure 2 Berlin Heart EXCOR (links: extrakorporale Lage, rechts: Implantationsprinzip)

### Short-term support

In acute heart failure, veno-arterial ECMO (extracorporeal membrane oxygenation) was implanted in patients with postcardiotomy heart failure, and as rescue therapy in patients with rapidly developing cardiogenic shock as bridge to long-term mechanical support or transplantation. In lung failure, veno-venous 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.




## Achievements 2007

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

## Collaborations:

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

## Selected references:


- Wilhelm MJ, Lachat ML, Prêtre R, Genoni M. Mechanische Kreislauf-unterstützung. In: Spitzenmedizin. Die Spitze des Eisbergs. Editor: V.E.Meyer. Kranich-Verlag, Zollikon, 2006.
- Wilhelm MJ, Lachat ML, Prêtre R, Zünd G, Salzberg S, Schmid ER, Ruschitzka F, Noll G, Genoni M. Management of decompensated severe heart failure: the axial-flow pump Berlin Heart INCOR for mechanical circulatory support. Kardiovaskuläre Medizin 2006;9(suppl 12):22S
- Husmann L, Leschka S, Salzberg SP, Lachat M, Genoni M, Marincek B, Alkadhi H. Sixty-four-slice computed tomographic coronary angiography in pseudoaneurysm of the ascending aorta: a useful modality to supplement the diagnosis. The Journal of thoracic and cardiovascular surgery. 2006;132, 2 e17-9
- Keller D, Wildermuth S, Boehm T, Boskamp T, Mayer D, Schuster HL, Marincek B, Alkadhi H. CT angiography of peripheral arterial bypass grafts: Accuracy and time-effectiveness of quantitative image analysis with an automated software tool. Academic radiology 2006; 13, 5 610-20
- Mayer D, Pfamatter T, Gauer JM, Wilhelm M, KLabler L, Weber D, Genoni M, Lachat M. Abdominal compartment syndrome after ruptured abdominal aortic aneurysm. European journal of vascular and endovascular surgery. 2006; 13 73-6
- Norton CK, Relf MV, Cox CW, Farley J, Lachat M, Tucker M, Murray J. Ensuring NCLEX-RN success for first-time test-takers. Journal of professional nursing. 2006; 22 5 322-6
- Simon RW, Lachat M, Pfammatter T, Amann-Vesti BR. Giant aneurysm of an aberrant right subclavian artery from the left aortic arch. The Journal of thoracic and cardiovascular surgery. 2006; 132 6 1478-9

- Wilhelm MJ, Lachat ML, Prêtre R, Zünd G, Salzberg S, Schmid ER, Ruschitzka F, Noll G, Genoni M. Management of decompensated severe heart failure: the axial-flow pump Berlin Heart INCOR for mechanical circulatory support. Kardiovaskuläre Medizin. 2006; 9 (suppl 12) 22
- Haas E, Meyer MR, Schurr U, Bhattacharya I, Minutti R, Nguyen HH, Heigl A, Lachat M, Genoni M, Barton M; Differential effects of 17beta-estradiol on function and expression of estrogen receptor alpha, estrogen receptor beta, and GPR30 in arteries and veins of patients with atheros clerosis. Hypertension 2007 Jun;49(6):1358-63. Epub 2007 Apr 23.
- Lachat M, Kucher N, Gauer JM, Mayer D, Pfammatter T, Wilhelm M, Genoni M. Coronary artery disease in abdominal aortic aneurysm patients; More Vascular and Endovascular Challenges; 2007; 137-145
- Lachat M, Labler L, Gauer JM, Mayer D, Genoni M, Pfammatter T. Traumatic aortic rupture: management and results. Controversies and Updates in Vascular Surgery; 2007; 308-314
- Lachat M, Kucher N, Gauer J, Mayer D, Pfammatter T, Wilhelm M, Genoni M. Coronary artery disease in abdominal aortic aneurysm patients. BIBA Publishing; More Vascular and Endovascular Chalenges; 2007: 137-45
- Lachat M, Kucher N, Gauer J, Mayer D, Pfammatter T, Wilhelm M, Genoni M. Coronary artery disease in abdominal aortic aneurysm patients. 2007: 24-31
- Lachat M, Labler L, Gauer J-M, Mayer D, Genoni M, Pfammatter T. Traumatic aortic ruptures: Management and Results. Minerva; Controversies and Updates in Vascular Surgery; 2007: 308-14
- Wilhelm MJ, Lachat ML, Prêtre R, Schmid ER, Ruschitzka F, Noll G, Genoni M. Mechanical circulatory support for terminal heart failure: experience with pulsatile and non-pulsatile flow devices. Kardiovaskuläre Medizin 2007;10(suppl 13):68S
- Wilhelm MJ. European results with the INCOR axial flow pump. La Pitié, 19.10.2008, invited talk.
- Wilhelm MJ. What's new in mechanical circulatory support? Invited talk, Swiss Society for Surgery, annual conference, Lausanne, 15.06.2007

## 2.1.4 Robotic Surgery and Innovative Technologies

PD Dr. med.
Jürg Grünenfelder

Dr. med.
André Plass

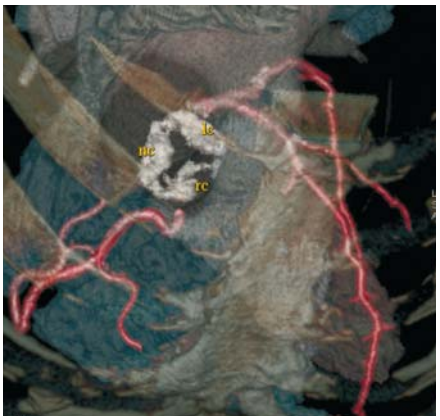
Dr. med.
Sacha Salzberg

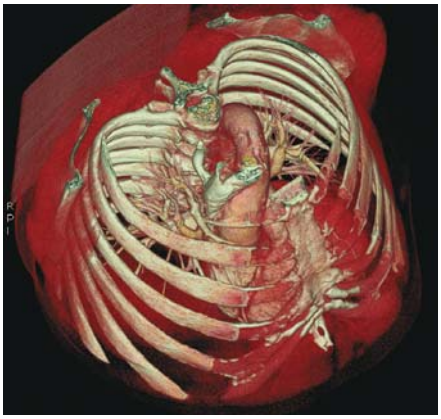
**Minimally invasive cardiac surgery**

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 2007

- EACTS European School CTS, 2007, André Plass, MD

## 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)

## Selected references:


- Accuracy of dual-source CT coronary angiography: First experience in a high pre-test probability population without heart rate control. Scheffel H, Alkadhi H, Plass A, Vachenauer R, Desbiolles L, Gaemperli O, Schepis T, Frauenfelder T, Schertler T, Husmann L, Grunenfelder J, Genoni M, Kaufmann PA, Marincek B, Leschka S. Eur Radiol. 2006 Dec;16(12):2739-47.
- Coronary artery imaging with 64-slice computed tomography from cardiac surgical perspective. Plass A, Grunenfelder J, Leschka S, Alkadhi H, Eberli FR, Wildermuth S, Zund G, Genoni M. Eur J Cardiothorac Surg. 2006 Jul;30(1):109-16.
- Aortic stenosis: comparative evaluation of 16-detector row CT and echocardiography. Alkadhi H, Wildermuth S, Plass A, Bettex D, Baumert B, Leschka S, Desbiolles LM, Marincek B, Boehm T. Radiology. 2006 Jul;240(1):47-55.
- Sixteen-channel multidetector row computed tomography versus coronary angiography in a surgical view. Plass A, Baumert B, Haussler A, Grunenfelder J, Wildermuth S, Eberli FR, Zund G, Genoni M. Heart Surg Forum. 2006;9(2):E572-8.
- Alkadhi H, Wildermuth S, Bettex DA, Plass A, Baumert B, Leschka S, Desbiolles LM, Marincek B, Boehm T. Mitral regurgitation: quantification with 16-detector row CT--initial experience. Radiology. 2006 238 2 454-63.
- Leschka S, Wildermuth S, Boehm T, Desbiolles L, Husmann L, Plass A, Koepfli P, Schepis T, Marincek B, Kaufmann PA, Alkadhi H. Noninvasive coronary angiography with 64-section CT: effect of average heart rate and heart rate variability on image quality. Radiology. 2006; 241 2 378-85.
- Matter CM, Rozenberg I, Jaschko A, Greutert H, Kurz DJ, Wnendt S, Kuttler B, Joch H, Grünenfelder J, Zund G, Tanner FC, Luscher TF. Effects of tacrolimus or sirolimus on proliferation of vascular smooth muscle and endothelial cells. Journal of cardiovascular pharmacology 2006;48 6 286-92.
- Plass A, Grünenfelder J, Genoni M. Reply to Lavi and Lavi. European journal of cardio-thoracic surgery. 2006; 30 5 814-15.

- Alkadhi H, Desbiolles L, Husmann L, Plass A, Leschka S, Scheffel H, Vachenauer R, Schepis T, Gaemperli O, Flohr TG, Genoni M, Marincek B, Jenni R, Kaufmann PA, Frauenfelder T; Aortic regurgitation: assessment with 64-section CT. Radiology. 2007 Oct;245(1):111-21. Epub 2007 Aug 23.
- Filsoufi F, Salzberg SP, Rahmanian PB, Schiano TD, Elsiesy H, Squire A, Adams DH; Early and late outcome of cardiac surgery in patients with liver cirrhosis. Liver Transpl. 2007 Jul;13(7):990-5.
- Plass A, Grünenfelder J, Reuthebuch O, Vachenauer R, Gauer JM, Zund G, Genoni M. New transverse plate fixation system for complicated sternal wound infection after median sternotomy. Ann Thorac Surg. 2007 Mar;83(3):1210-2.
- Rahmanian PB, Castillo JG, Salzberg S, Sanz J, Filsoufi F. Computed tomography angiography is an excellent tool for the diagnosis of congenital coronary artery anomalies: a report of 2 cases. Heart Surg Forum. 2007;10(2):E99-E101.
- Scheffel H, Leschka S, Plass A, Vachenauer R, Gaemperli O, Garzoli E, Genoni M, Marincek B, Kaufmann P, Alkadhi H. Accuracy of 64-slice computed tomography for the preoperative detection of coronary artery disease in patients with chronic aortic regurgitation.
  Am J Cardiol. 2007 Aug 15;100(4):701-6. Epub 2007 Jun 21.

## 2.1.5 Congenital Heart Surgery

Prof Dr. med.
Rene Pretre

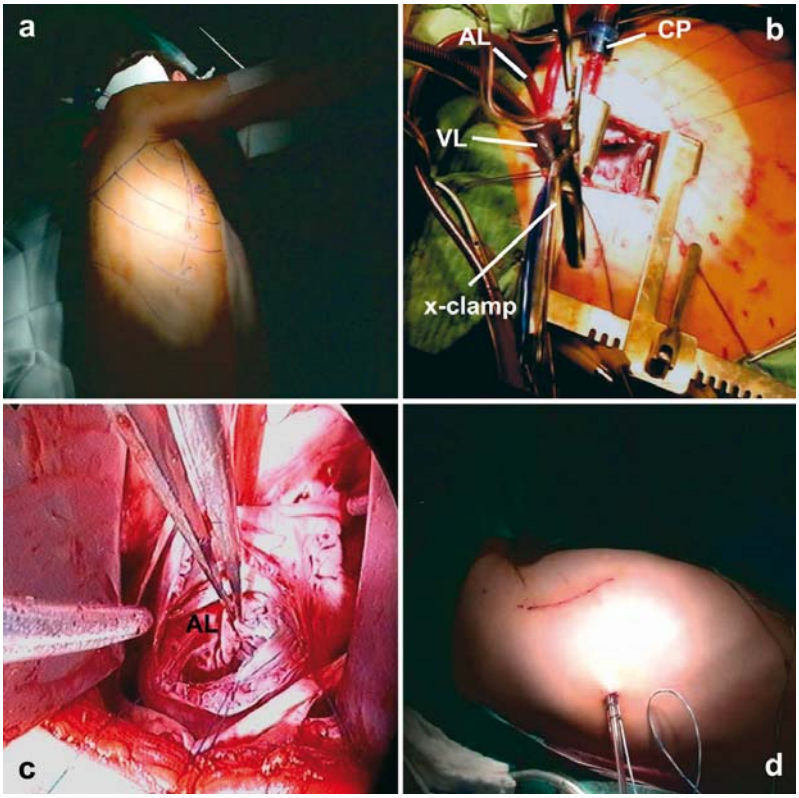
PD Dr. med.
Ali Dodge-Khatami

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 2007

- 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

## Selected references:


- Kadner A, Dodge-Khatami A, Dave H, Knirsch W, Bettex D, Pretre R. Closure of restrictive ventricular septal defects through a right axillary thoracotomy. Heart Surg Forum. 2006; 9(6):E836-9
- Dodge-Khatami A, Buchel EV, Knirsch W, Kadner A, Rousson V, Dave HH, Bauersfeld U, Pretre R. Brain natriuretic peptide and magnetic resonance imaging in tetralogy with right ventricular dilatation. Ann Thorac Surg. 2006 Sep;82(3):983-8.
- Dave H, Dodge-Khatami A, Kadner A, Pretre R. Modified technique for heterotopic implantation of a right ventricular outflow tract conduit. Ann Thorac Surg. 2006 Jun;81(6):2321-3.
- Dodge-Khatami A, Deanovic D, Sacher P, Weiss M, Gerber AC. Clinically relevant tracheomalacia after repair of esophageal atresia: the role of minimal intra-operative dissection and timing for aortopexy. Thorac Cardiovasc Surg. 2006 Apr;54(3):178-81.
- Pretre R, Khatchatourov G, Kadner A, Genoni M. Application and adjustment of artificial chordae to the mitral valve using an approach through the aortic valve. Ann Thorac Surg. 2006 Aug;82(2):761-2.
- Pretre R, Kadner A, Dave H, Bettex D, Genoni M. Tricuspidisation of the aortic valve with creation of a crown-like annulus is able to restore a normal valve function in bicuspid aortic valves. Eur J Cardiothorac Surg. 2006 Jun;29(6):1001-6. Epub 2006 May 3.
- Dave HH, Buechel ER, Pretre R. Muscle-sparing extrapleural approach for the repair of aortic coarctation. Ann Thorac Surg. 2006 Jan;81(1):243-8.

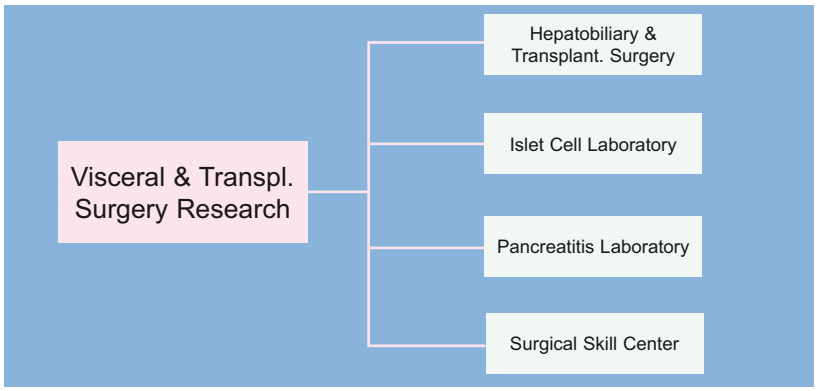
- A Dodge-Khatami, M Rahn, R Prêtre, U Bauersfeld. Dual chamber epicardial pacing for the failing atriopulmonary Fontan patient. Ann Thorac Surg. 2006 80(4):1440-4.
- NC Aellig, C Balmer, A Dodge-Khatami, M Rahn, R Prêtre, U Bauersfeld. Long-term follow-up after pacemaker implantation in neonates and infants. Ann Thorac Surg. 2006 Epub ahead of print.
- U Bauersfeld,M Tomaske, A Dodge-Khatami, M Rahn, CI Kellenberger, R Prêtre. Initial experience with implantable cardioverter defibrillator systems using epicardial and pleural electrodes in paediatric patients. Ann Thorac Surg. 2006 Epub ahead of print
- A. Dodge-Khatami, C.L. Backer, M. Meuli, R. Prêtre, M. Tomaske, C.Mavroudis; Migration and colon perforation of intraperitoneal cardiac pacemaker systems. The annals of thoracic surgery; 2007, 83: 2230-2
- A. Dodge-Khatami, M. Schmid, V. Rousson, M. Fasnacht, C. Döll, U. Bauersfeld, R. Prêtre; Risk factors for reoperation after relief of congenital subaortic stenosis. Interactive cardiovascular and thoracic surgery; 2007; 6 (Suppl 3): 255
- A. Dodge-Khatami, S. Tschuppert, C. Doell, R. Arlettaz, O. Baenziger, V. Rousson, R. Prêtre; The effect of ductal diameter on surgical and medical closure of patent ductus arteriosus in preterm neonates: size matters. Cardiology in the young ; 2007; 17-Suppl. 1: 20
- A. Lianos, K. Waldvogel, L. Molinari, A. Dodge-Khatami, M. Kaufmann, U. Bauerfeld, B. Latal; Predictors of preoperative neurological outcome and its impact on children with congenital heart disease undergoing car diopulmonary bypass. Cardiology in the young; 2007; 17-Suppl. 1: 99
- Aellig NC, Balmer C, Dodge-Khatami A, Rahn M, Pretre R, Bauersfeld U; Long-term follow-up after pacemaker implantation in neonates and infants. The annals of thoracic surgery; Ann Thorac Surg. 2007 Apr;83(4):1420-3.
- Bauersfeld U, Tomaske M, Dodge-Khatami A, Rahn M, Kellenberger CJ, Prêtre R. Initial experience with implantable cardioverter defibrillator systems using epicardial and pleural electrodes in pediatric patients. Ann Thorac Surg. 2007 Jul;84(1):303-5.
- C. Doell, A. Dodge-Khatami, M. Fasnacht, B. Frey, O. Baenziger; Hemodynamic and oxygenation changes after oral Sildenafil for pulmonary hypertension in infants and children. Cardiology in the young; 2007; 17-Suppl; 1:75
- D. Deanovic, A.C. Gerber, A. Dodge-Khatami, C.M. Dillier, M. Meuli, M. Weiss; Tracheoscopy assisted repair of tracheo-esophageal fistula (TARTEF): a 10-year experience.; Paediatr Anaesth. 2007 Jun;17(6):557-62
- Dodge-Khatami A, Knirsch W, Tomaske M, Prêtre R, Bettex D, Rousson V, Bauersfeld U. Spontaneous closure of small residual ventricular septal defects after surgical repair. Ann Thorac Surg. 2007 Mar;83(3):902-5.
- Kadner A, Fasnacht M, Kretschmar O, Prêtre R. Traumatic free wall and ventricular septal rupture - ‚hybrid' management in a child. Eur J Cardio-thorac Surg, 2007 May;31(5):949-51. Epub 2007 Mar 6.

- Kadner A, Tulevski II, Bauersfeld U, Prêtre R, Valsangiacomo-Buechel ER, Dodge-Khatami A. Chronic pulmonary valve insufficiency after repaired tetralogy of Fallot: diagnostics, reoperations and reconstruction possibilities. Expert Rev Cardiovasc Ther. 2007 Mar;5(2):221-30.
- Leschka S, Oechslin E, Husmann L, Desbiolles L, Marincek B, Genoni M, Prêtre R, Jenni R, Wildermuth S, Alkadhi H. Pre- and postoperative evaluation of congenital heart disease in children and adults with 64-section CT.
  Radiographics 2007 May-Jun;27(3):829-46.
- Leschka S, Scheffel H, Desbiolles L, Plass A, Gaemperli O, Valenta I, Husmann L, Flohr TG, Genoni M, Marincek B, Kaufmann PA, Alkadhi H. Image quality and reconstruction intervals of dual-source CT coronary angiography: recommendations for ECG-pulsing windowing. 2007; 42: 543-9
- M. Greutmann, P. Trigo-Trindade, O. Kretschmar, A. Dodge-Khatami, R. Prêtre, U. Bauersfeld. Conversion of atrio-pulmonary connection to total cavo-pulmonary connection: a single centre experience with the Fontan circulation. Cardiology in the young; 2007, 17 – Suppl; 1: 98
- M. Tomaske, B. Gerritse, L. Kretzers, R. Prêtre, A. Dodge-Khatami, M. Rahn, U. Bauersfeld. Long-term follow up of bipolar steroid-eluting epicardial pacing leads in children. Cardiology in the young; 2007, 17 – Suppl; 1: 36
- M. Tomaske, P. Harpes, A. Dodge-Khatami, N. Amacker, U. Bauersfeld. Do threshold trend fluctuations of epicardial leads predict pacing and sensing characteristics?
  Cardiology in the young; 2007, 17 – Suppl; 1: 75
- M. Tomaske, P. Harpes, A. Dodge-Khatami, U. Bauersfeld. Long-term experiences of AutoCapture controlled pacing in children. Cardiology in the young; 2007, 17 – Suppl; 1: 51
- M. Tomaske, P. Harpes, R. Prêtre, A. Dodge-Khatami, U. Bauersfeld. Evolution of paced QRS and QTc intervals in children with epicardial pacing leads. Evolution of paced QRS and QTc intervals in children with epicardial pacing leads. Clin Res Cardiol. 2007 Nov;96(11):787-93. Epub 2007 Aug 13.
- Stolzmann P, Scheffel H, Bettex D, Karlo C, Frauenfelder T, Prêtre R, Marincek B, Alkadhi H. Subvalvular aortic stenosis: comprehensive cardiac evaluation with dual-source computed tomography.
  J Thorac Cardiovasc Surg. 2007 Jul;134(1):240-1, 241.e1.
- Tomaske M, Harpes P, Pretre R, Dodge-Khatami A, Bauersfeld U. Long-term experience with AutoCapture-controlled epicardial pacing in children. Europace. 2007 Aug;9(8):645-50. Epub 2007 Jul 13.
- U. Bauersfeld, M. Tomaske, A. Dodge-Khatami, M. Rahn, C.J. Kellenberger, R. Prêtre. Initial experience with implantable cardioverter defibrillator systems using epicardial and pleural electrodes in pediatric patients. Ann Thorac Surg. 2007 Jul;84(1):303-5.
- U. Bauersfeld, M. Tomaske, A. Dodge-Khatami, M. Rahn, C.J. Kellenberger, R. Prêtre. Short term follow up of implantable cardioverter defibrillator systems using epicardial and pleural electrodes in pediatric patients. Cardiol Young, 17-Suppl. 1:36-37

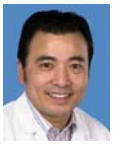
## 2.2 Visceral & Transplant Surgery Research

PD Dr. phil II
Rolf Graf

Prof. Dr. med.
Pierre-Alain Clavien




### 2.2.1 Hepatobiliary & Transplant Surgery



PD Dr. med.
Yinghua Tian

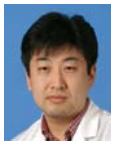
Dr. med.
Panco Georgiev

Dr. med.
Antonio Nocito

Dr. med.
Ashraf Osman

Dr. med.
Stefan Heinrich

Dr. med.
Andreas Rickenbacher



Dr. sc. nat.
Jae-Hwi Jang

Udo Ungethüm

**Ischemia / Reperfusion Injury and Liver Transplantation**
PD Dr. med. Yinghua Tian, Dr. med. Antonio Nocito, Dr. Jae-Hwi Jang, Dr. med. Ashraf Osman, Dr. med. Panco Georgiev, Dr. med. Stefan Heinrich, Dr. med. Andreas Rickenbacher

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

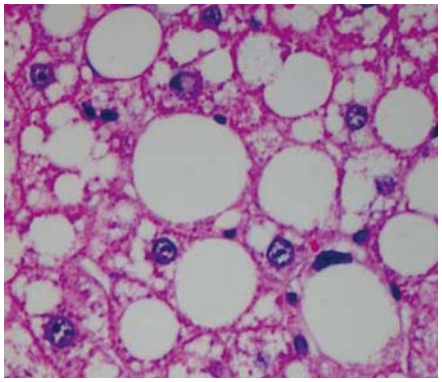
**Hepatoprotective effects of Omega-3 Fatty Acids**

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.

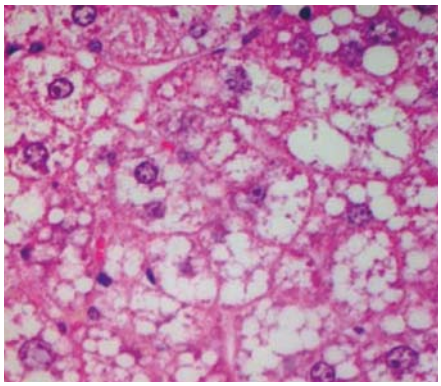
We have previously shown that dietary supplements containing n-3 polyunsaturated fatty acids (PUFAs) normalize the abnormally high n-6: n-3 PUFA ratio in the macrosteatotic liver of ob/ob mice and enhance their tolerance to I/R injury. In the lean liver, thromboxane A2 (TXA2), an eicosanoid derived from arachidonic acid (AA), notoriously contributes to hepatic microvascular failure and hepatocellular injury after I/R. (El Badry et al; 2 Got Hepatology)

Two groups of ob/ob mice were fed either a standard laboratory chow or fish oil (a source of n-3 PUFAs) enriched diet for 12 weeks. Hepatic content of n-6 PUFAs was measured. A model of partial (70%) hepatic ischemia was applied

for 45 minutes. Hepatic microcirculation and plasma levels of TXA2 in the suprahepatic vena cava were investigated. Hepatocellular injury was assessed by plasma levels of alanine aminotransferase (ALT).
Dietary supplementation with n-3 PUFAs resulted in a pronounced reduction in hepatic content of arachidonic acid. After ischemia and 30 minutes of reperfusion, a significant rise in the vasoactive TXA2 levels was observed in the control diet fed animals with a remarkable decrease of the sinusoidal red blood cell velocity and volumetric blood flow. Supplementation with n-3 PUFAs resulted in consistent reduction of TXA2 levels after reperfusion. Microcirculation parameters were significantly ameliorated. Concurrently, ALT levels disclosed pronounced reduction in n-3 PUFAs supplemented animals. The impact of TXA2 on I/R injury of the macrosteatotic liver was further highlighted by demonstrating a similar protection when control diet fed animals were treated with a selective TXA2 receptor antagonist.



Macrosteatosis



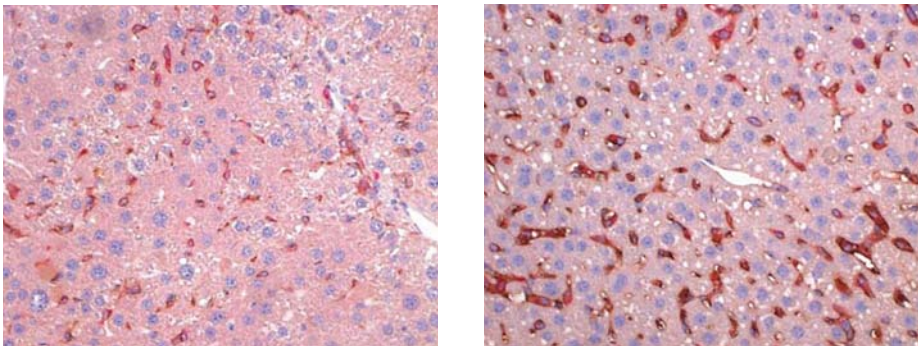
Microsteatosis

**Cholestasis**

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. In addition, a model of partial bile duct obstruction was developed. 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 quantified. These experiments provide a road-map for pathophysiological events during cholestasis. Further experiments are under way to investigate the role of fibrotic mediators on liver regeneration and tumor progression.
(Georgiev et al., 2007, Gut)

**Pharmacological preconditioning in cirrhotic mouse livers**

Ischemic preconditioning is the pre-emptive proven strategy to reduce ischemic injury in the liver, but it can be harmful in the elderly or patients with liver diseases. Ischemic preconditioning induces a protective effect via activation of an oxidative stress response. We hypothesized that Fas ligand and tumor necrosis factor-α can induce a similar response. Therefore, we tested if these death ligands could mimic ischemic preconditioning.
Death ligands were given 40 minutes before ischemia which was then performed for 60 minutes in cirrhotic mice.
To elucidate the mechanism, we used zinc protophorphyrin, an inhibitor of heme oxygenase-1 and gadolinium chloride, an inhibitor of Kupffer cells.
Compared to the control group, death ligand preconditioning strongly reduced all markers of injury: serum transaminase levels, necrosis and apoptosis. Preconditioning caused an up-regulation of heme oxygenase-1, predominantly in macrophages. When zinc-protophorphyrin or gadolinium chloride was applied prior to preconditioning, the beneficial effect of preconditioning was lost.
These results demonstrate that ischemic preconditioning can be replaced by death ligand preconditioning in the cirrhotic liver to prevent ischemic injury. The protective mechanism is depending on heme oxygenase-1 induction in macrophages. These results open doors for novel hepato-protective strategies in liver surgery and transplantation (Jang et al., Gut, *in press*).



Preconditioning | no preconditioning

Kupffer cell staining of mouse livers with and without death ligand preconditioning.

**Platelets and platelet-derived serotonin promote tissue repair in the mouse liver**

Hepatic ischemia and reperfusion (I/R) 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 promotes liver regeneration. We hypothesized that platelets may contribute to reperfusion injury and repair after normothermic hepatic ischemia. Therefore, we assessed the impact of platelets in normothermic hepatic I/R injury using models of impaired platelet function and immune thrombocytopenia. Inhibition of platelet function in mice was achieved via clopidogrel feeding. Immune thrombocytopenia was induced via intraperitoneal injection of anti-CD41 antibody. Platelet-derived serotonin was investigated using mice lacking tryptophan hydroxylase 1. Mice were subjected to 60 minutes of partial hepatic ischemia and various time points of reperfusion. Hepatic injury was determined via AST and histological analysis of the necrotic area as well as leukocyte infiltration. Liver regeneration was determined via proliferating cell nuclear antigen and Ki67 immunohistochemistry. Neither inhibition of platelet function nor platelet depletion led to a reduction of I/R injury. Liver regeneration and repair were significantly impaired in platelet-depleted animals. Mice lacking peripheral serotonin were deficient in hepatocyte proliferation, but otherwise displayed normal tissue remodeling. Therefore we conclude that platelets have no direct impact on the pathogenesis of normothermic I/R 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 remodelling (Nocito et al., 2007, Hepatology).

**Serotonin in the pathophysiology of nonalcoholic steatohepatitis**

Nonalcoholic steatohepatitis (NASH) is one of the most common causes of liver enzyme elevation in the West. Its prevalence is likely to increase further, paralleling the epidemic increase of the metabolic syndrome. Serotonin degradation by monoamine oxidase A (MAO-A) was recently implicated as an important source of reactive oxygen species. We therefore tested the pathogenetic role of serotonin in a murine model of diet-induced steatohepatitis. Wild-type and serotonin-deficient, tryptophan hydroxylase 1 knock out mice (Tph1(-/-) were fed a choline-methionine-deficient diet for 2 and 6 weeks. MAO-A was inhibited by administration of chlorgyline.
After choline-methionine-deficient diet, Tph1(-/-) mice displayed an equal degree of steatosis, yet reduced hepatocellular injury and less severe inflammation. The difference in these NASH-defining features could be attributed to an increased uptake and catabolism of serotonin, yielding enhanced levels of reactive oxygen species and lipid peroxides, which mediated hepatocellular injury by mitochondrial damage and inflammation.

Inhibition of MAO-A reduced hepatocellular damage in wild-type mice. Correspondingly, MAO-A expression was up-regulated significantly in human NASH.
This study provides evidence that serotonin plays a role in the pathogenesis of steatohepatitis, and therefore might represent a novel target for the prevention and treatment of NASH (Nocito et al., 2007, Gastroenterology).

## Achievements 2007

Scientific

- Demonstration that low-dose death ligands can have a cytoprotective effect in cirrhotic livers
- Serotonin is a key molecule in the development of non-alcoholic-steato-hepatitis
- Thromboxane release in the fatty liver accelerates ischemic injury

Personnell

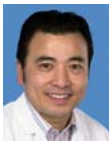
- Dr. A. El-Badry has won the SGC award from the Swiss Society of Surgery

## Collaborations:

- PD Dr. W. Jochum, Institut für Klinische Pathologie, UniversitätsSpital Zürich
- Prof. A. Pietrangelo, University of Modena, Italy

## Selected references:


- El-Badry AM, Moritz W, Contaldo C, Tian Y, Graf R, Clavien PA. Prevention of reperfusion injury and microcirculatory failure in macrosteatotic mouse liver by omega-3 fatty acids. Hepatology 2007;45:855-63.
- El-Badry AM, Graf R, Clavien PA. Omega 3 - Omega 6: What is right for the liver? J Hepatol 2007;47:718-25.
- Nocito A, Dahm F, Jochum W, Jang JH, Georgiev P, Bader M, Renner EL, Clavien PA. Serotonin mediates oxidative stress and mitochondrial toxicity in a murine model of nonalcoholic steatohepatitis. Gastroenterology 2007;133:608-18.
- Nocito A, Georgiev P, Dahm F, Jochum W, Bader M, Graf R, Clavien PA. Platelets and platelet-derived serotonin promote tissue repair after normo-thermic hepatic ischemia in mice. Hepatology 2007;45:369-76.



PD Dr. med.
Yinghua Tian

Dr. med.
Katarzyna Furrer

Dr. med.
Christopher Soll

## Liver regeneration

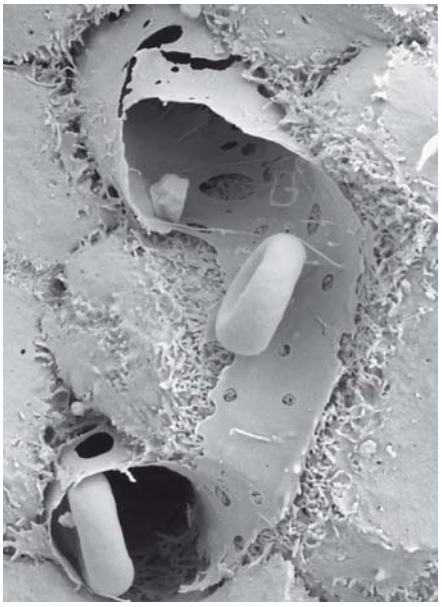
Dr. med. K. Furrer, Dr. med. Christopher Soll, PD Dr. med. Yinghua Tian

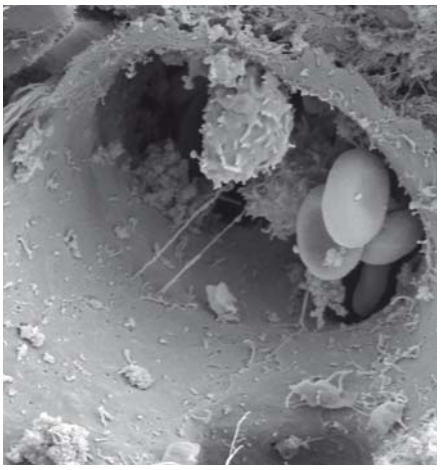
### Regeneration in the aging liver

The increasing age of patients with liver disease requiring surgery leads to more complications related to failure of regeneration. Previous work demonstrated that the aging liver has a reduced capacity to regenerate after major tissue loss. In addition to molecular changes, it has been observed that the sinusoidal structure is severely affected in older individuals. A loss of fenestration in the sinusoidal endothelial cells (SEC) might reduce the flow of blood components and soluble mediators into the space of Disse resulting in impaired signalling. Serotonin has recently been implicated in the early process of regeneration in young mice. We therefore hypothesize that serotonin may influence fenestration and propagate access to the parenchyma after tissue loss.

We investigated mice of different age (up to 2 years). In contrast to young mice, the SEC of old mice exhibited very few fenestrae. Consistent with these changes, regeneration was impaired in old mice. During regeneration, the expression of serotonin receptor mRNA was highly increased in young but not old livers ($p<0.0004$). Pre-treatment of old mice with DOI, a serotonin receptor agonist, significantly increased fenestration size and diameter of SEC similar to the young phenotype. Subsequently, hepatectomy in the DOI treated old mice disclosed improved regeneration as demonstrated by increased numbers of proliferating hepatocytes. Furthermore, these animals had a better survival after hepatectomy

We conclude that serotonin improves regeneration in old mice by increasing SEC fenestration. Pharmacological targeting of serotonin receptors in the liver may provide a novel approach to improve surgical interventions in the aging population.

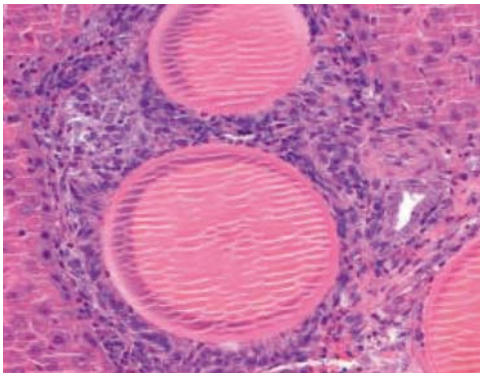




Scanning electron microscopy of young (left) and old (right) mouse livers showing fenestration (young) and defenestration (old) of the endothelial layer.

## 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.
As previously shown, hepatectomy induced a high increase in proliferation of hepatocytes. In contrast, regeneration was less intense in ligated animals and even more diminished in embolized livers. Concurrent with embolization of the sinusoids, we observed a foreign body reaction which included attraction of a high number of macrophages. We conclude that in contrast to current believe ligation is more effectively inducing regeneration than embolization (Furrer et al., *in press*, Hepatology).



### Achievements 2007

Scientific
- Serotonin rescues regeneration in the old liver.
- Induction of liver regeneration is superior in ligated compared to embolized livers.

Personnel
- Dr. K. Furrer received the research award from the Association of Research in Surgery (ARS, Swiss Society of Surgery)

### Collaborations:

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

PD Dr. med.
Philipp Dutkowski

Dr. med.
Olivier de Rougemont

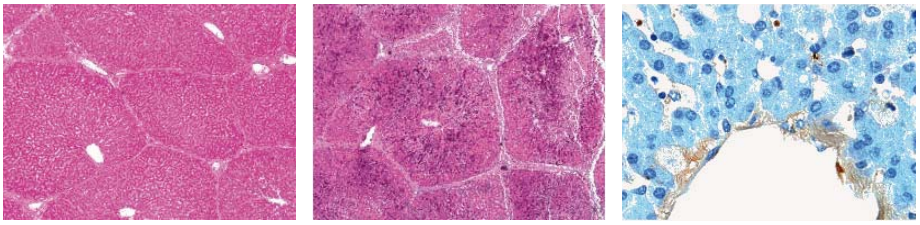
## Hypothermic oxygenated pig liver perfusion

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

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.

In a further attempt to reach a clinically acceptable system for livers, comparable in size and architecture to human, we designed and constructed a perfusion system in which pig or human livers can be maintained. Pig livers were obtained from a slaughterhouse and perfused for several hours with diluted blood. Fresh resected livers with minimal cold storage served as controls with normal bile flow and low cytosolic enzyme release. Pig livers after warm ischemia (1 hr) and extended cold storage (7 hrs) exhibited considerable loss of function while hypothermic oxygenated perfusion (HOPE) for 1 hr after warm ischemia and cold storage reversed hepatocyte and endothelial cell injury.

We now attempt to prove our results in a large animal transplant study to bring this strategy to human.



HE stainings of pig liver sections with (left) or without (middle) HOPE prior to extended cold storage and reperfusion (middle). vWF staining after HOPE treatment (right).

### Achievements 2007

- 42 experiments with isolated perfusion of the pig liver
- SNF grant: Hypothermic oxygenated perfusion of non heart beating donors prior to transplantation

### Selected references:


- Dutkowski P, de Rougemont O, Clavien PA. Machine perfusion for “marginal” liver grafts. American J Transpl 2008 (in press).
- Dutkowski P, Graf R, Clavien PA. Rescue of the cold preserved liver by hypothermic oxygenated machine perfusion. American J. Transpl. 2006; 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.
Olivier de Rougemont

Dr. med.
Christopher Soll

Dr. med.
Antonio Nocito

Dr. med.
Felix Dahm

cand. med.
Oliver Fisher

**Oncology**

Dr. med. Olivier de Rougemont, Dr. med. Christopher Soll, Dr. med. Antonio Nocito, Dr. med. Felix Dahm, cand. med. Oliver Fisher

**Mitochondrially targeted ceramide LCL-30 inhibits colorectal cancer**

The sphingolipid ceramide is intimately involved in the growth, differentiation, senescence, and death of normal and cancerous cells. Mitochondria are increasingly appreciated to play a key role in ceramide-induced cell death. Recent work showed the C16-pyridinium ceramide analogue LCL-30 to induce cell death in vitro by mitochondrial targeting. The aim of the current study was to translate these results to an in vivo model. We found that LCL-30 accumulated in mitochondria in the murine colorectal cancer cell line CT-26 and reduced cellular ATP content, leading to dose- and time-dependent cytotoxicity. Although the mitochondrial levels of sphingosine-1-phosphate (S1P) became elevated, transcription levels of ceramide-metabolising enzymes were not affected. In mice, LCL-30 was rapidly absorbed from the peritoneal cavity and cleared from the circulation within 24 h, but local peritoneal toxicity was dose-limiting. In a model of subcutaneous tumour inoculation, LCL-30 significantly reduced the proliferative activity and the growth rate of established tumours. Sphingolipid profiles in tumour tissue also showed increased levels of S1P. In summary, we present the first in vivo application of a long-chain pyridinium ceramide for the treatment of experimental metastatic colorectal cancer, together with its pharmacokinetic parameters. LCL-30 was an efficacious and safe agent. Future studies should identify an improved application route and effective partners for combination treatment. (Dahm et al., 2007, Br J Cancer).

**Human hepatocellular cancer-cells survive with serotonin**

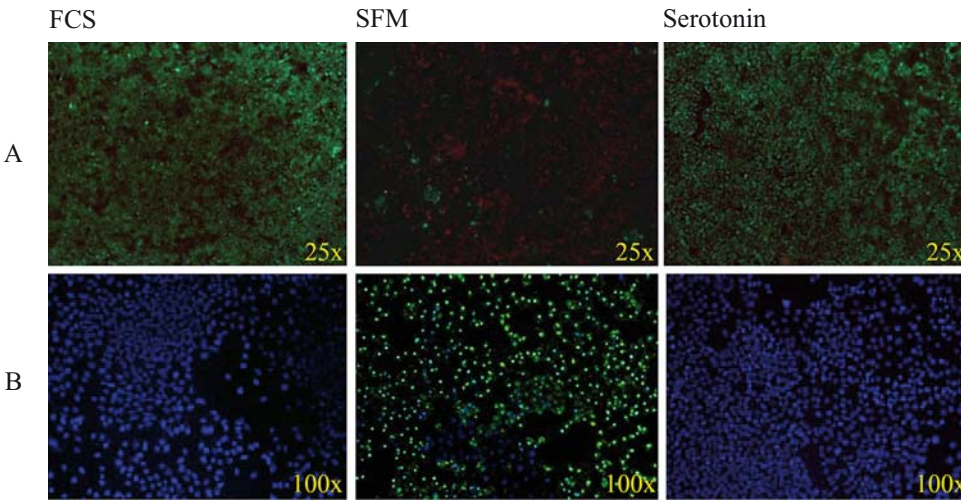
Serotonin, a neurotransmitter and vascular active substance, can act as a potent cellular mitogen on different cell types and is crucial for liver regeneration. Involvement in the tumour biology of lung and prostate cancer has been described. Therefore, we wanted to evaluate the impact of serotonin in hepatocellular carcinomas.

$^{3}$H-thymidin-incorporation was measured in three different human hepatocellular cancer cell-lines (HepG2, Huh7, Hep3B) after stimulation with serotonin (5-HT). To distinguish between proliferation and improved survival a combined calcein/ethidium-staining was performed on these cells. Immunoblots were used to investigate serotonin-dependent pathways leading to survival of the cell-lines.

$^{3}$H-thymidin-incorporation indicated an increased proliferation of serum-starved HepG2, Huh7 and Hep3B with 100 µM 5-HT compared to serum-free-media after 48h ($p=0.01$). Interestingly, calcein/ethidium-staining shows a similar amount of living cells stimulated either with 10% serum or 100 µM 5-HT after serum-starvation, whereas untreated cells were predominant positive for ethidium indicating cell death. Immunoblots revealed a correlation between activation of the kinases PKC and ERK1/2 after stimulation with serotonin-dependent prevention of cell death.

Immunohistochemistry for the serotonin receptor HTR1B and HTR2B were performed on a tissue-micro-array from 61 patients with hepatocellular carcinomas. Of the 61 hepatocellular carcinomas, 14% and 26% were positive for HTR1B and HTR2B, respectively. There was a significant correlation with the proliferation marker Ki67 and HTR2B. Receptor-staining of HTR1B was associated with vascular invasion of the tumor.

This is the first study that proposes a role of serotonin in the tumor biology of hepatocellular carcinomas. Our data suggest serotonin as a survival factor for hepatocellular cancer cell lines. Furthermore, more than one-fourth of the patients with hepatocellular carcinoma were positive for HTR2B. Hence serotonin may represent a novel target for the treatment of hepatocellular cancer, especially as numerous safe and effective serotonin-targeting drugs are in clinical use today.




48 h serum starvation of Huh7 cells followed by 48h culture with 10 % fetal calf serum (FCS), serum free media (SFM) or 100 µM serotonin. A: Overlay of Calcenin and Ethidium stainings. B: Overlay of Hoechst and TUNEL stainings.

**Non-invasive imaging of subcutaneous tumors in mice**

The analysis of various aspects of hepatocellular carcinoma (HCC) in mouse experimental models can be very demanding. The intrahepatic growth and localisation of metastasis is usually assessed by histological examination, which is labour-intensive and only semi-quantitative at best. Therefore a HCC cell line which is easy to track by non-invasive imaging modalities could be of benefit for research purposes. We developed a hepatocellular carcinoma mouse cell line, which stably expressed two fluorescing reporter proteins, DsRed (red fluorescence) and EGFP (green fluorescence). The reporter genes were introduced into the HCC cells by lentiviral transduction. In vivo imaging of subcutaneously implanted cells was performed by the use of the IVIS® Imaging System 200 Series (Xenogen™). Both reporter expressing cell lines were detectable after subcutaneous injection into nude mice in quantities as low as 100'000 cells. While DsRed detection was facilitated by a considerably low background signal, autofluorescence within the green spectrum resulted in an unfavourable signal/background ratio which affected the sensitivity in detecting EGFP expressing cells. However, luciferase expressing cells and measurement of bioluminescence after luciferin injection proved to be the most sensitive detection method for in vivo imaging. By our initial results, the proof of concept of non-invasive imaging by generating fluorescent/bioluminescent reporter expressing cell lines could be demonstrated. In terms of sensitivity related to low background signals, the luciferase reporter system was superior to the fluorescent based models.

## Achievements 2007

Scientific

- Colorectal cancer growth can be inhibited in vivo by ceramides
- Serotonin acts as a survival factor for cancer cells
- Established non-invasive *in vivo* imaging in mice

## 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
- Prof. Dr. M. Pruschy, Radio-Oncology, 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, Bielawska A, Nocito A, Georgiev P, Szulc ZM, Bielawski J, Jochum W, Dindo D, Hannun YA, Clavien PA. Mitochondrially targeted ceramide LCL-30 inhibits colorectal cancer in mice. Br J Cancer 2008 (in press).

## 2.2.2 Islet Cell Laboratory

Dr. phil. II
Wolfgang Moritz

PD Dr. med.
Markus Weber

med. pract.
Patrick Kugelmeier

Lu Tuyet Trinh

**Size does matter – an approach to improve human islet 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 Endocrinolology 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 independent 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 extent within the islet core regions. Animal studies and observations from clinical islet transplantations suggest that islet transplants composed of mainly small islets exhibit improved graft function as compared to transplants consisting of mainly large islets (Lehmann et al. 2007).
We recently developed a method to generate islets of small dimensions originating from isolated pancreatic islets of rats (Cavallari et al. 2007). We could demonstrate that the so called “pseudoislets” were indistinguishable from intact islets in terms of their cellular composition and architecture with no central necrosis present, as occasionally observed in intact predominantly large islets. Small pseudoislets showed an improved glucose dependent insulin secretion capacity which was enhanced by 2.5-fold when compared to size matched intact islets. To test whether this approach is also feasible for clinical islet transplantation, human islets isolated from donor organs were dispersed into single cells and reaggregated into pseudoislets of defined small dimensions by the so called hanging drop method. Analogous to our observations with rat islets, the newly formed pseudoislets were similarly composed and arranged as compared to native islets. The insulin secretory capacity of small pseudoislets reached 88% after 6 days and 160% after 14 days of reaggregation culture compared to a mixed population of intact fresh islets. Surprisingly, the biphasic secretion pattern typical for human islets was restored as represented by an augmentation of the first phase insulin secretion.

This could in part be explained by the fact that the total insulin content expressed per islet equivalent in small islets, both native and pseudoislets, was 3-4 fold higher than compared to large islets.
The superior biological function with respect to the total insulin content, first phase insulin secretion, and stimulation index identifies small human pseudoislets as an ideal source for the improvement of graft function in islet transplantation. Provided our in vitro observations can be confirmed in a transplant setting, this approach could well be transferred into clinical practice.

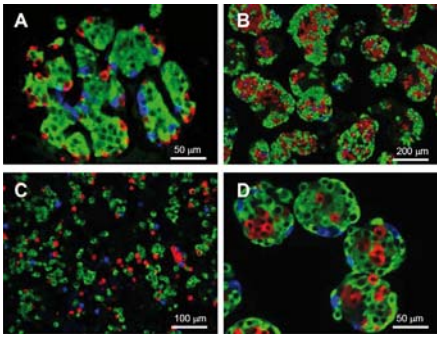



Figure 1: Pictures show the relative distribution of the three main cell types, insulin producing β-cells (green), glucagon producing α-cells (red) and Somatostatin producing δ-cells (blue) in (**A**) native islets within the human pancreas, (**B**) isolated human islets, (**C**) single cell population after enzymatic dissociation, and (**D**) in pseudoislets after 7 days of reaggregation.

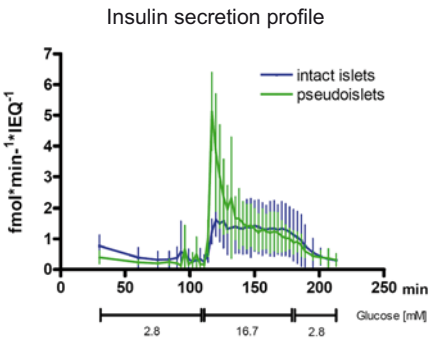



Figure 2: Insulin secretion profile at low and high glucose. A mixed population of fresh, intact human islets was compared to human pseudoislets composed of 250 cells/islet after 14 days of reaggregation. Note the improved first phase insulin secretion shortly after raising glucose concentration from 2.8 mM to 16.7 mM, which only occurs in pseudoislets.

## Achievements 2007

- Pseudoislets can be formed from islolated human islets derived from donor organs
- In vitro generated human pseudoislets show an enhanced functionality, represented by an increased and more physiological insulin secretion pattern.

## Collaborations:

- The research project is also part of a close collaboration with Dr. Züllig and PD Dr. Lehmann 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 lead by Prof. Hoerstrup.

## Selected references:


- Cavallari G, Zuellig RA, Lehmann R, Weber M, Moritz W. Rat pancreatic islet size standardization by the „hanging drop“ technique. Transplant Proc. 2007; 39(6): 2018-20
- Lehmann R, Zuellig RA, Kugelmeier P, Baenninger PB, Moritz W, Perren A, Clavien PA, Weber M, Spinas GA. Superiority of small islets in human islet transplantation. Diabetes 2007; 56(3):594-603

## 2.2.3 Pancreatitis Research Laboratory

PD Dr. phil. II
Rolf Graf

Dr. med.
Li K. Sun

Dipl. phil. II
Theresia
Reding Graf

Martha Bain

PD Dr. med.
Daniel Bimmler

Dr. sc. nat.
Alberto Silva

cand.med.
Soo-Young Kim

**$PGE_2$ modulates cytokine regulation in the pancreas**

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

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. COX-2 activity leads to increased production of the prostaglandins $PGE_2$ which has both pro-inflammatory and cell-protective activities.

We could demonstrate that in both, the rat and human pancreas, receptors for $PGE_2$ are present and some of the four isoforms were highly increased during chronic inflammation. Based on this we localized the receptors to the acinar cells and to some infiltrating cells. In the rat model of chronic pancreatitis (WBN/Kob) receptor expression was increased in comparison to control rats.

Stimulation of acinar cells with $PGE_2$ in combination with TNFα led to a synergistic increase in synthesis and secretion of cytokines and chemokines. This process was protein kinase A dependent. We conclude that targeting prostaglandin pathways may be a strategy to interfere with inflammation.

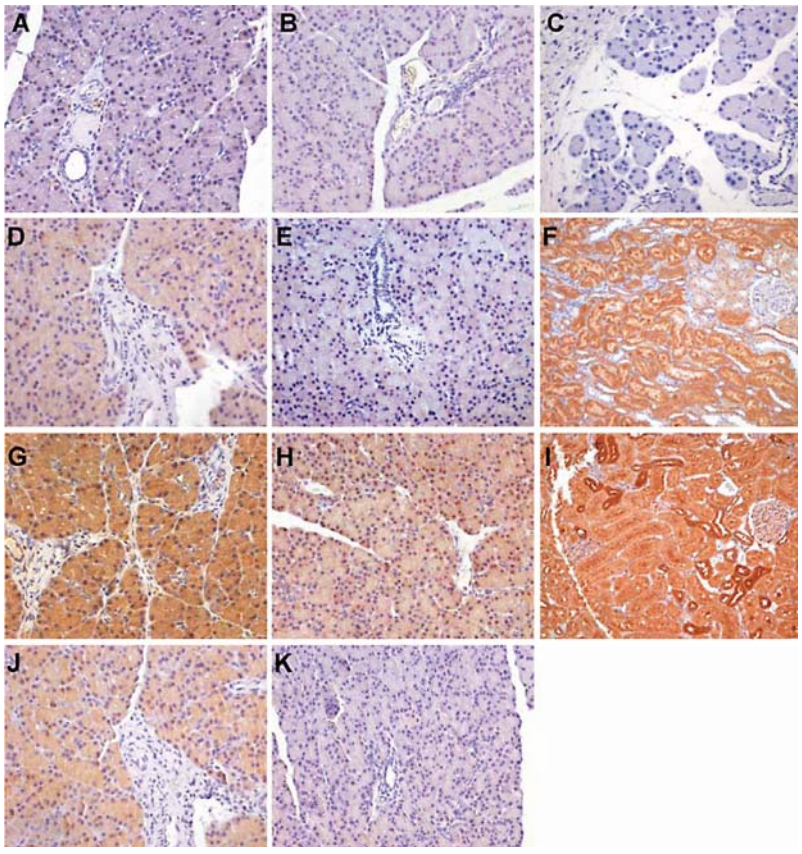



Figure 1: Prostanoid receptor (EP1-4) localization by immunohistochemistry in the WBN/Kob rat pancreas. Prostanoid receptor localization in WBN/Kob (A,D,G, J) and Wistar (B, E, H, K) rat pancreas specimen. A-B: EP1; D-E: EP2; G-H: EP3; J-K EP4. F, I: sections from kidney using antibodies against EP1 and EP3 respectively. C: Control section stained by using normal rabbit IgG.

**Activity of COX-2 in chronic inflammation in the mouse**
Dr. A. Silva, M. Bain, PD Dr. R. Graf

Our efforts to investigate the role of COX-2 in chronic pancreatic inflammation led us to establish a new model in the mouse. With this model we can use knock-out and transgenic mice to investigate the relationship between COX-2 and inflammation, fibrosis and repair. In pilot experiments, we evaluated whether acute pancreatitis was affected by the lack of COX-2. In young mice, inflammation was reduced, however, in older animals this effect was lost. In chronic inflammation, there were no differences concerning cytokine and chemokine expression. Thus, the absence of COX-2 apparently did not change the inflammatory activity. However, repair mechanisms seemed to be impaired in COX-2 knock-out animals, suggesting that prostaglandins are involved in tissue repair in the mouse pancreas.

**Interaction of PSP/reg and PAP with stellate cells**
PD Dr. med. D. Bimmler, PD Dr. R. Graf

Pancreatic stone protein (PSP/reg) and pancreatitis-associated protein (PAP) are secretory stress proteins highly up-regulated during inflammation. Previous reports suggested that these proteins may interact with cell surface proteins of inflammatory cells. We hypothesized that they may also be involved during fibrogenesis and tested this by determination of interactions with human pancreatic stellate cells (PSC). Activated stellate cells produce collagen, secrete matrix-metallo-proteases (MMP) and tissue inhibitors of MMPs (TIMPs). These proteins are tightly regulated and coordinate extracellular matrix formation.
We could demonstrate that PSP/reg inhibits cell migration of PSC, thus negatively influencing fibrogenesis.

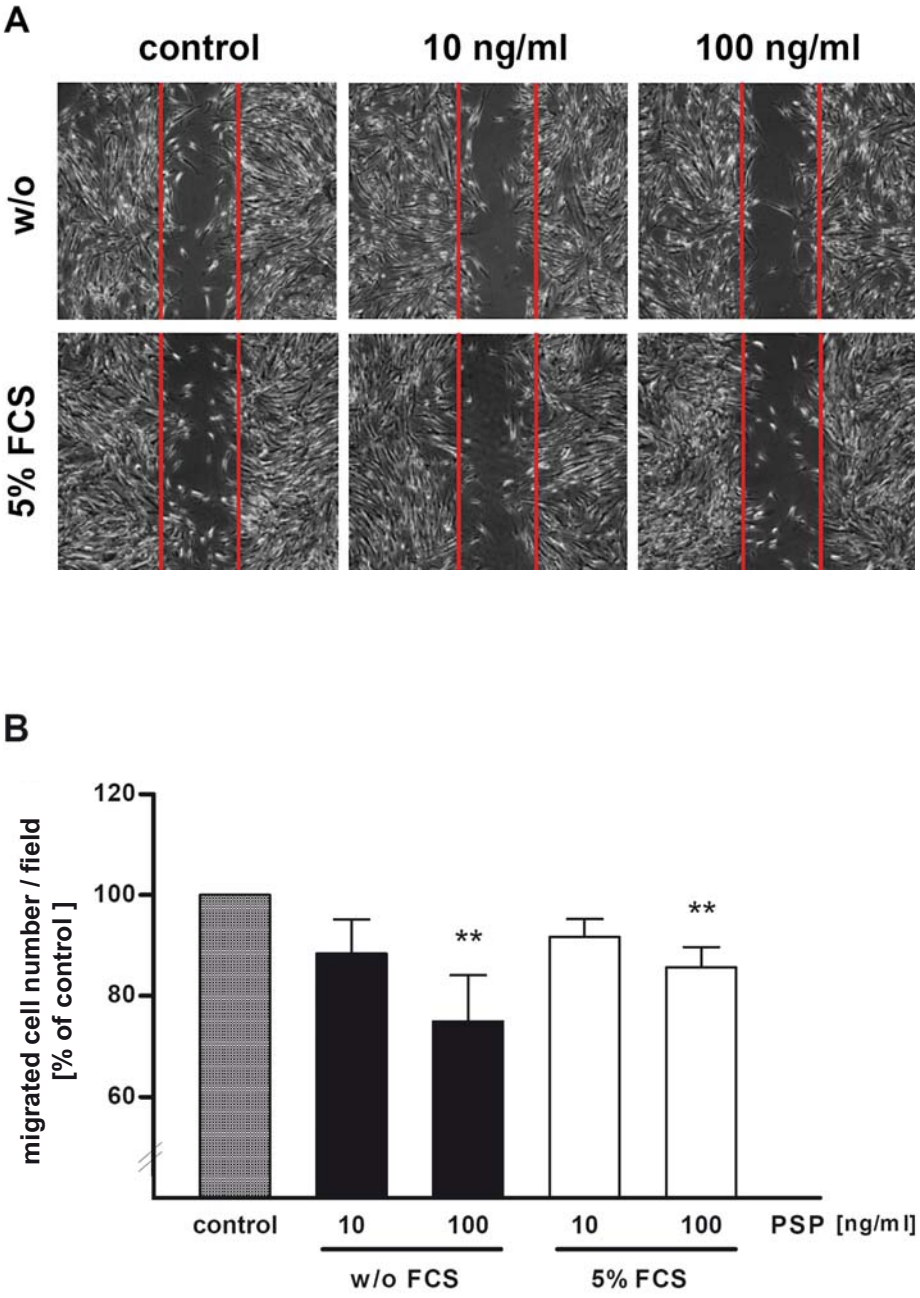



Figure 2. Effect of PSP/reg on migration of cultured human PSC. PSC were seeded in 24-well plates and starved overnight when reaching confluence. The cell layer was scratched with a 10 µl pipette tip. Then PSC were maintained in medium without or with 5% FCS. After the addition of 10 and 100 ng/ml PSP/reg for 24 hours, cultures were stopped and phase contrast micrographs were taken. Migrated cells were counted inside the boundary of wound area. (A) One representative experiment. (B) Data are expressed as means ± SD of 3 independent experiments, each condition was performed in triplicate culture wells. Statistically significant difference (**P < 0.01) compared with control.

## Achievements 2007

Scientific

- We localized PGE2 receptors in pancreatic tissue.
- Publication in the American Journal of Physiology.
- Establishment of chronic inflammation model in the mouse pancreas.

## Collaborations:

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

## Selected references:


- Sun LK, Reding T, Bain M, Heikenwalder M, Bimmler D, Graf R. Prostaglandin E2 modulates TNF-alpha-induced MCP-1 synthesis in pancreatic acinar cells in a PKA-dependent manner. Am J Physiol Gastrointest Liver Physiol 2007;293:G1196-204.
- Reding T, Bimmler D, Perren A, Sun LK, Fortunato F, Storni F, Graf R. A selective COX-2 inhibitor suppresses chronic pancreatitis in an animal model (WBN/Kob rats): significant reduction of macrophage infiltration and fibrosis. Gut 2006;55:1165-73.

## 2.2.4 **Surgical Skill Center**

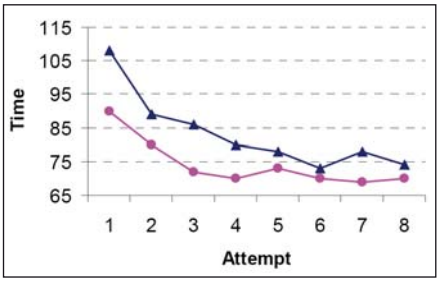
Clin. Ass. Prof.
PD Dr. med.
Dieter Hahnloser

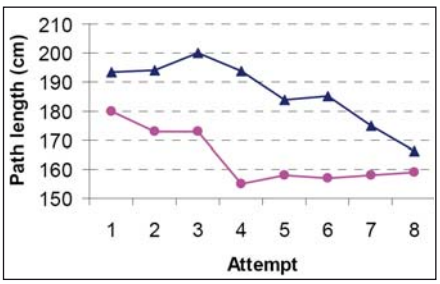
PD Dr. med. Dieter Hahnloser

The goal of surgical skills training should be for all individuals to be competent upon completion of training while not spending wasteful hours performing repetitive motions without further benefit. Therefore, there is a need for proficiency-based VR training programs where the performance of a group of experts defines the level of skills that the trainee must reach and achieve constantly before progressing to the operating room.

We investigated the performance parameters of 307 participants of the 19th to 22nd Davos International Gastrointestinal Surgery Workshops (2002-2005). We demonstrated that specific performance measurements in a standardized task on a virtual reality simulator can objectively discriminate between different level of trainees in laparoscopic surgery; that the simulator performance assessment correlates with a structured evaluation of performance in a pelvitrainer laparoscopic task and correlates with an expert's evaluation of performance during an exercise of conventional surgery (Rosenthal R, Gantert WA, Hamel C, Hahnloser D et al. J Laparoendosc Adv Surg Tech A 2007).

In a pilot study 12 residents and 6 consultants performed 5 basic and 4 advanced laparoscopic exercises on the LapMentorTM eight times each. Results demonstrated that for camera navigation residents reached expert levels after 5 attempts and for the clip and cut exercise barely after 8 attempts (Figure). Therefore, for the first exercise 5 attempts and reaching constantly expert time seems adequate, but for the second exercise 8 attempts seem insufficient. The next step is now to compare experts with different levels of trainees on various virtual reality tasks, to establish benchmark criteria for each task.

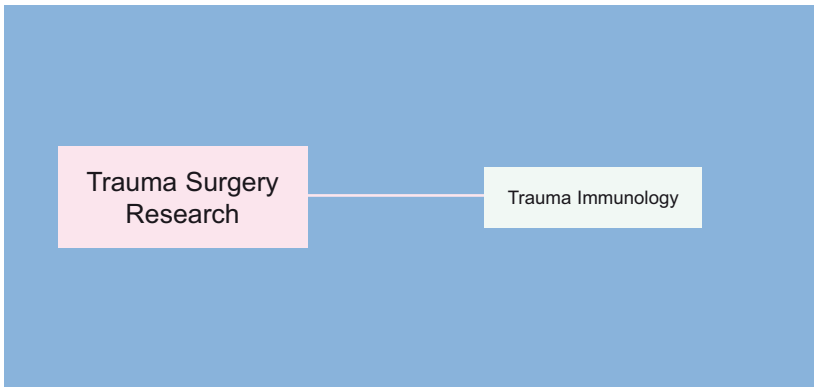


## 2.3 Trauma Surgery Research


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.
Emanuel Benninger


Dr. med.
Philipp Lenzlinger


Dr. med.
Ladislav Mica


Dr. med.
Ludwig Labler


Dr. med.
Thomas Lustenberger


Dr. med.
Mario Rancan


Ursula
Steckholzer

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

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

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

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

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

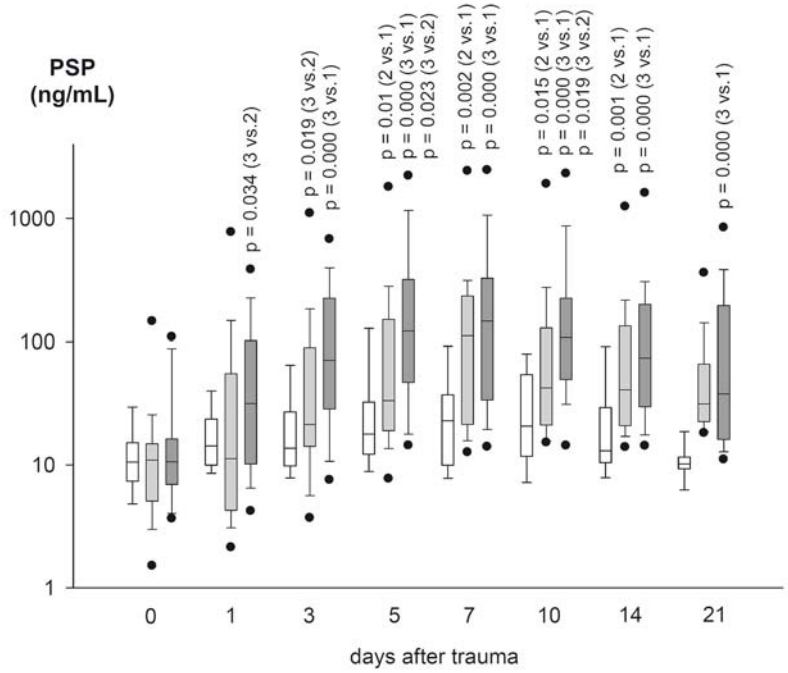



Fig. 1. Kinetics of PSP/reg serum levels in polytrauma patients. PSP/reg serum levels from polytrauma patients without infection (1, white bars, n= 18), with local infection (2, grey bars, n= 32) or sepsis (3, darkgrey bars, n= 33) were measured by ELISA from day of admission until day 21. Data is given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

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

## Achievements 2007

- Participation in the worldwide largest trauma F7 Trauma-1711 phase III study: “A multi-center, randomized, double-blind, parallel group, placebo controlled trial to evaluate the efficacy and safety of activated recombinant factor VII (rFVIIa/NovoSeven®/NiaStase®) in severely injured trauma patients with bleeding refractory to standard treatment”. The Division of Trauma Surgery, University Hospital Zürich with Dr. M. Keel as the principle investigator (PI) is the worldwide leading recruitment site with 21 patients recruited so far.

Kongressbeiträge:

- L. Mica, L. Labler, O. Trentz, L. Härter, M. Keel. Increased survival of neutrophil granulocytes in VAC®-treated compared to Epigard®-treated wounds. 1st EATES/ETS 23-26. May 2007, Graz, Austria
- T. Lustenberger. L. Mica, M. Turina, O. Trentz, M. Keel. Traumatic brain injury increases mortality and morbidity in patients with hemorrhagic shock. 1st EATES/ETS 23-26. May 2007, Graz, Austria
- T. Lustenberger, L. Mica, L. Labler, O. Trentz, M. Keel. Outcome and microbiological analysis during sepsis after severe trauma. 1st EATES/ ETS 23-26. May 2007, Graz, Austria
- E. Benninger, L. Labler, O. Trentz, M. Menger, C. Meier. In vitro comparison of intraabdominal hypertension development after different temporary abdominal closure techniques. 1st EATES/ETS 23-26. May 2007, Graz, Austria
- T. Lustenberger. L. Mica, M. Turina, O. Trentz, M. Keel. Severe hemorrhagic shock drastically increases mortality in patients with traumatic brain injury. 94th SGC Congress, 13-15.06. 2007 Lausanne, CH
- L. Mica, L. Labler, L. Härter, O. Trentz, M. Keel. VAC®-Therapy induces local activation of neutrophil granulocytes in traumatic wounds. 94th SGC Congress, 13-15.06. 2007 Lausanne, CH
- M. Turina, A. Billeter, L Mica, T. Lustenberger, O Trentz, M Keel. Serum Procalcitonin and Interleukin-6 Correlate with Infectious Complications in 1079 Severely Traumatized Patients, with Strongest Correlations Observed in Subsequently Septic Patients. 94th SGC Congress, 13-15.06. 2007 Lausanne,CH
- E. Lucchinetti, S. Ambrosio, J. Aguirre, T. Meier, L. Härter, M. Zaugg. Sevoflurane at sedative doses prevents endothelial dysfunction and activation of leukocytes after ischemia/reperfusion injury in humans and modulates mRNA expression in peripheral blood. 6th Day of Clinical Research 1-2.03. 2007, ZKF, ZH, CH
- D. Mayer, T. Pfammatter, J. Gauer, M. Wilhelm, L. Labler, D. Weber, M. Genoni, M. Lachat. Solving the problem of abdominal compartment syndrome after endovascular aortic aneurysm repair for ruptured abdominal aortic aneurysms: Literature Review, 6th Day of Clinical Research 1-2.03. 2007, ZKF, ZH, CH
- L. Mica, L. Labler, O. Trentz, L. Härter, M. Keel. Increased survival of neutrophil granulocytes in VAC-treated compared to Epigard-treated wounds. 6th Day of Clinical Research 1-2.03. 2007, ZKF, ZH, CH
- L. Härter, L. Labler. L. Mica, O. Trentz, M. Keel. VAC-therapy induces local activation of neutrophil granulocytes in traumatic wounds. 6th Day of Clinical Research 1-2.03. 2007, ZKF, ZH, CH
- E. Benninger, M. Laschke, M. Cardell, O. Trentz, M. Menger, C. Meier. In vivo comparison of intra-abdominal pressure development after different temporary abdominal closure techniques. 11. Chirurgische Forschungstage, 15.-17.11.2007 Saarbrücken, Germany

- E. Benninger, M. Laschke, M. Cardell, O. Trentz, M. Menger, C. Meier. Measurement of compartment pressure of the rectus sheat during intraabdominal hypertension: Validation and comparison with established intraabdominal pressure measurement techniques in a porcine model Bretschneider Preisträger Sitzung, 11. Chirurgische Forschungstage, 15.-17.11.2007 Saarbrücken, Germany
- E. Benninger, L. Labler, O. Trentz, M. Menger, C. Meier. In vitro comparison of intraabdominal hypertension development after different temporary abdominal closure techniques. 3rd World Congress on Abdominal Compartment Syndrome 22.-24.3.2007 Antwerpen, Belgium
- T. Lustenberger. L. Mica, O. Trentz, M. Keel. Timing for definitive osteosynthesis of orthopaedic trauma after initial damage control surgery. 93th AAST meeting 27.-30.09.2007 Las Vegas USA

**Collaborations:**

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

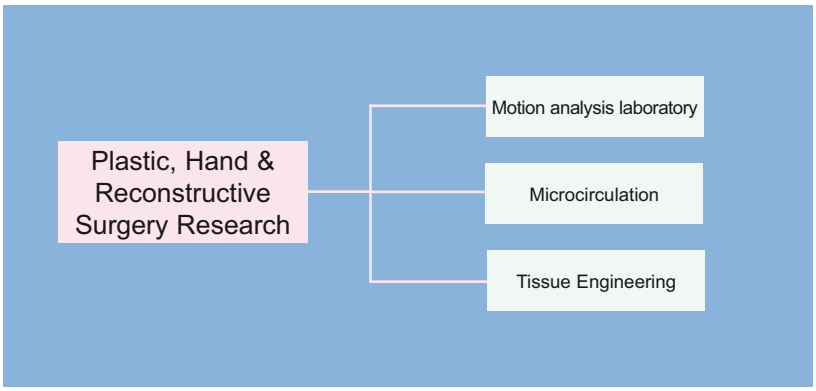
**Selected references:**


- Von Känel R, Hepp U, Kraemer B, Traber R, Keel M, Mica L, Schnyder U (2007) Evidence for low-grade systemic proinflammatory activity in patients with posttraumatic stress disorder. J Psychiat Res, 41:744-52.
- Lucchinetti E, Ambrosio S, Aguirre J, Herrmann P, Härter L, Keel M, Meier T, Zaugg M (2007) Sevoflurane inhalation at sedative concentrations provides endothelial protection against ischemia-reperfusion injury in humans. Anesthesiology, 106:262-8.
- Keel M, Meier C. (2007) Chest injuries - what is new? Curr Opin Crit Care, 13:674-9.
- Lucchinetti E, Aguirre J, Feng J, Zhu M, Suter M, Spahn DR, Härter L, Zaugg M. (2007) Molecular evidence of late preconditioning after sevoflurane inhalation in healthy volunteers. Anesth Analg, 105(3):629-40.
- Grosjean MB, Lenzlinger PM, Stahel PF, Yatsiv I, Shohami E, Trentz O, Kossmann T, Morganti-Kossmann MC. (2007) Immunohistochemical characterization of Fas (CD95) and Fas Ligand (FasL/CD95L) expression in the injured brain: relationship with neuronal cell death and inflammatory mediators. Histol Histopathol, 22(3):235-50
- Rancan M, Esser MP, Kossmann T. (2007) Irreducible traumatic obturator hip dislocation with subcapital indentation fracture of the femoral neck: a case report. J Trauma, 62(6):E4-6.
- Zwahlen RA, Labler L, Trentz O, Grätz KW, Bachmann LM. (2007) Lateral impact in closed head injury: a substantially increased risk for diffuse axonal injury--a preliminary study. J Craniomaxillofac Surg, 35(3):142-6.
- Labler L, Trentz O. (2007) The use of vacuum assisted closure (VAC(trade mark)) in soft tissue injuries after high energy pelvic trauma. Langenbecks Arch Surg, 392(5):601-9.

## 2.4 Plastic, Hand & Reconstructive Surgery Research

Prof. Dr. med.
Pietro Giovanoli,

Dr. med.
Maurizio Calcagni

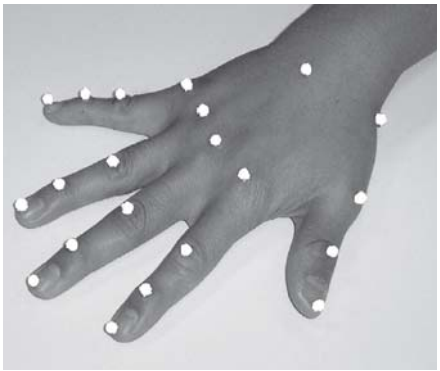



### 2.4.1 Motion analysis laboratory

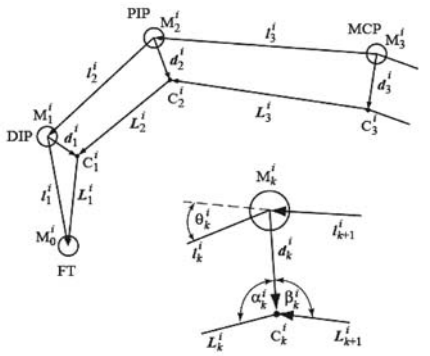
Dr. med.
Maurizio Calcagni

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

The quantitative in vivo assessement of wrist and small finger joints kinematics is of paramount importance to understand posttraumatic and pathological processes. We use a set of markers which allows us to analyze motion 3-D during flexion, extension, pronation and supination.



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




Geometrical model characterizing the relation between skin markers and the real internal rotation centres. During long finger flexion and extension ( by Zhang et al. , 2003)

To fix the rotation centre for marker positioning we use the algorithm of Zhang, based on a geometrical model, whereby the relationsship between local movement of skin markers (Mki) and the real rotation centres (Cki) during finger flexion/ extension is taken into consideration. The superfialvectors (lki ) are characterized by varying lengths during motion, whereas the internal vectors remain stable (Lki )konstant.

## Achievements 2007

Talks:

- Invited Speaker at the XIIth FESSH Congress, Panel on “Fingertip injuries: reconstruction possibilities” with a presentation on “Double palmar and dorsal flaps for fingertip reconstruction”, Athens (Greece), 27-30.06.07
- Congress organization
  29th Congress of the Deutschsprachigen Arbeitsgemeinshaft für Mikrochirurgie der peripheren Nerven und Gefäße (DAM), Zurich (CH) 22-24.11.2007
- Scientific Director
  Scientific Director of the 3rd Zurich Workshop on Hand Flaps. A cadaver dissection Course, Institut of Anatomy, University of Zurich, Zurich (Switzerland), 01-02.03.2007

## Collaborations:

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

## Selected references:


- A. Atzei, M. Calcagni, B. Breda, G. Fasolo, G. Pajardi, L. Cugola: Clinical evaluation of a hyaluronan-based gel following microsurgical reconstruction of peripheral nerves of the hand. Microsurgery 27: 2-7, 2007
- A. Biraima, M. Steinemann, K. Käch, M. Calcagni: Carpal Arthrodesis with the Limited Wrist Fusion Plate (Hub Cup). Proceedings of the 10th Congress of International Federation of Societies for Surgery of the hand, Sydney (Australia), march 11-15, 2007
- M. Calcagni1, A. Biraima, K. Käch, P. Giovanoli: The 2 mm locking compression plates (lcp) in the treatment of the fractures of the first metacarpal bone: indications and results. Abstract of the 45° National Congress of Italian Society of Hand Surgery, Napoli, 03-06.10.2007
- B. Fiedel, A. Biraima, A. R. Jandali, M. Calcagni: Ergebnisse nach operativer Versorgung von Läsionen der plamaren Platte des proximalen Interphalangealgelenkes der Fingern. Abstracts of the 41th Congress of the Swiss Society for Hand Surgery, Lucerne (CH), November 2007
- M. Calcagni, A. Biraima, C. Hess, I. Tami, P. Giovanoli: Scaphotrapeziotrapezoidal joint arthroplasty with a pyrocarbon spacer (STPI). Abstracts of the 41th Congress of the Swiss Society for Hand Surgery, Lucerne (CH), November 2007

## 2.4.2 Microcirculation

Dr. med.
Claudio Contaldo

Dr. med.
Nicole Lindenblatt

Dr. med.
Ahmed Elsherbiny

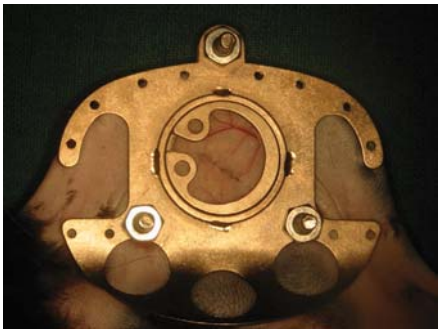
Dr. med.
Sebastian Vetter

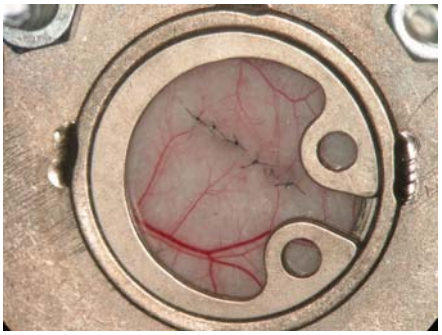
Chen Fanfan
PhD Student

cand. med.
Dominik Högger

**Improved healing in hypercholesteremic murine wounds after EPO treatment**
Dr. med. Claudio Contaldo, Dr. med. Nicole Lindenblatt, Dr. med. Ahmed Elsherbiny, Dr. med. Sebastian Vetter, cand. med. Dominik Högger, Dr. med.Maurizio Calcagni and Prof. Dr. Pietro Giovanoli

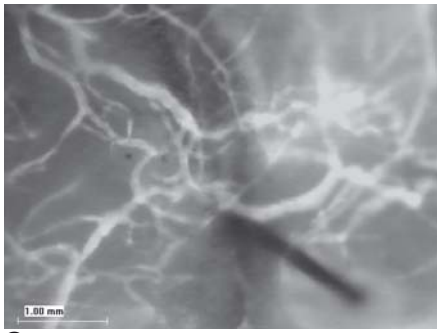
Inadequate blood supply is the most common cause of altered wound healing representing a considerable source of morbidity in daily plastic surgery practice. Erythropoietin (EPO) is intensively investigated for its nonhematopoietic vasculoprotective effects. The present study demonstrates the potential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



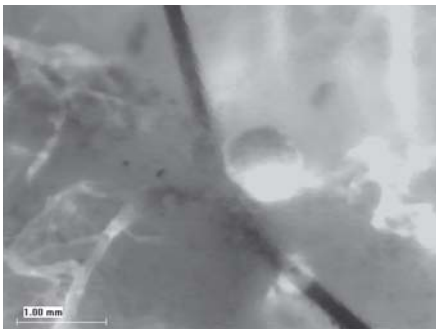


The dorsal skinfold chamber allows to monitor quantitatively angiogenic hemodynamics in an incisional wound in vivo.

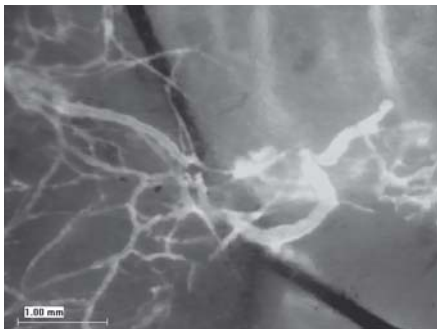

A




C




B



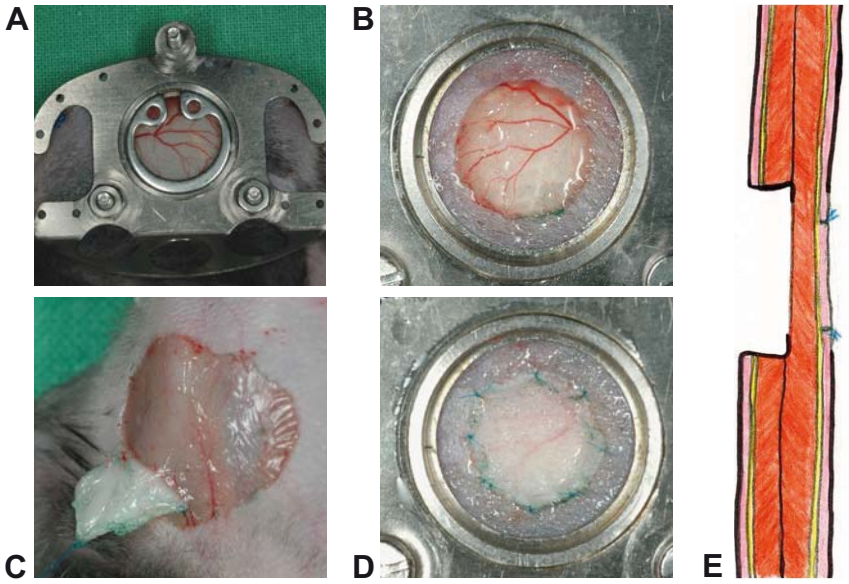

D

Images demonstrate the functional neovessel density in normal healing mice 1(A), 3 (B), 7 (C), and 10 days (D) after the creation of an incisional wound in a dorsal skinfold chamber. 9-0 Nylon suture is shown.

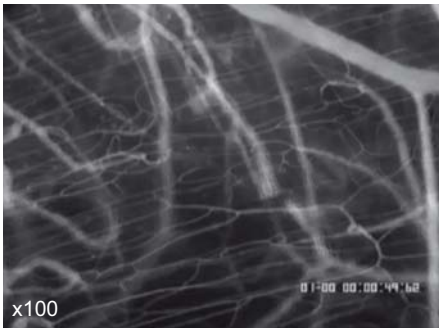
**A new model to study the revascularization of skin grafts in vivo – the role of angiogenesis**

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

Existing models of skin graft revascularization are mostly based on histological evaluations lacking the possibility to observe vascular biology in vivo. Aim of this study was therefore to develop a new animal model allowing for continuous monitoring of the microcirculation of wound bed and graft.
Skin and subcutaneous tissue were removed in a circular area from the back of dorsal skinfold chamber preparations in mice leaving only the layer of striated muscle and subcutaneous vessels as wound bed. A respective full-thickness skin graft was harvested form the groin and sutured into the defect in the back of the chamber. Intravital microscopy was performed at baseline and at day 1-5 and 10.
Capillary widening of the bed appeared at day 1 post grafting and increased until day 4. Capillary buds and sprouts firstly appeared at day 2. Initial filling of graft capillaries occurred at day 3 resulting in almost complete restoration of the original pattern of skin microcirculation at day 5. There was evidence for induction of temporary angiogenic response in the graft most probably due to ingrowing vessels from the bed on day 4 and 5.
This new model allows for simultaneous observation of bed and skin graft at repetitive time points and for subsequent in vitro studies. It provides favourable in vivo conditions to further delineate the exact mechanism of vessel connection and the complex process of angiogenesis in this context as well as to study the vascularization of tissue-engineered skin substitutes.

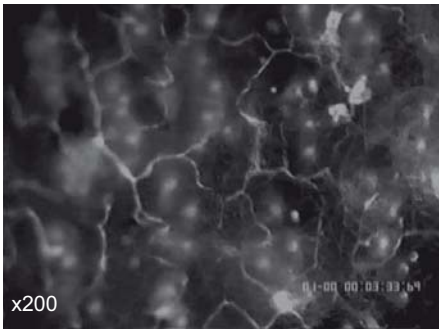



The dorsal skinfold chamber was chosen as a basis for the new model (A). Preparation of the wound bed by removal of the skin and most of the subcutaneous tissue in the back of the chamber (B). Harvesting of the skin graft from the groin of the animal (C). Transfer of the graft to the defect in the back of the chamber, which is fixed with 8 single sutures (D). The profile sketch of the skinfold chamber (front=left margin) illustrates that the skin graft (pink) fits well into the defect on the back and that both wound bed and graft can easily be accessed by intravital micoscopy (E).

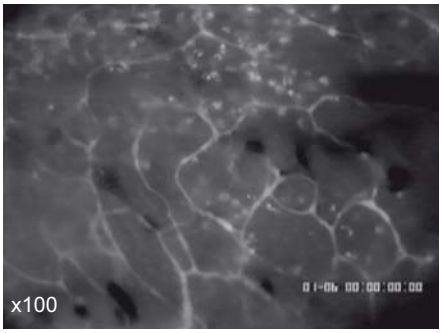



A


C




B



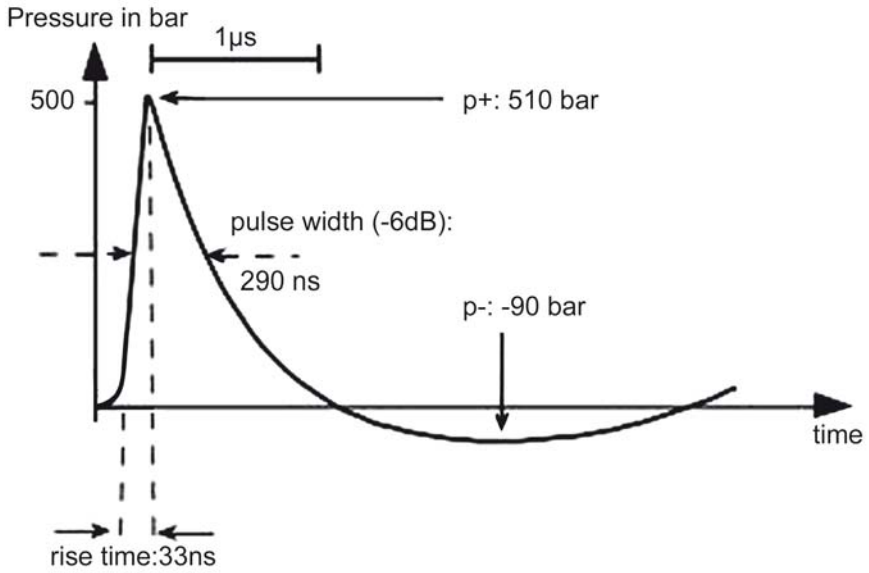

D

Intravital microscopic imaging of microvascular perfusion pattern in the wound bed (A) and the graft (B) at baseline. After 72 hours of skin grafting we observed progressing angiogenesis in the wound bed with budding and sprouting (C). Within the graft vasculature a slow blood flow appeared, demonstrating a pattern which was comparable to that of the original microvasculature of the skin (D). Magnification x100-200.

**Effects of Extracorporeal Shock Wave Energy on Microcirculation of Healthy Mice**

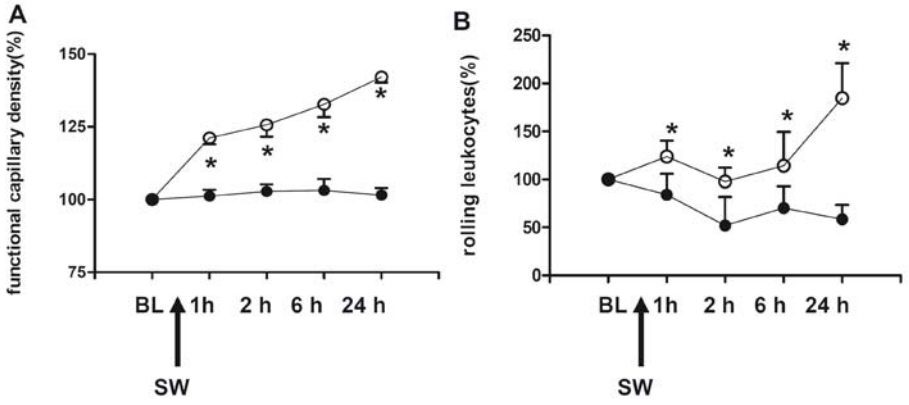
Dr. med. Claudio Contaldo, Chen Fanfan, Dr. med. Nicole Lindenblatt, Dr. med. Sebastian Vetter, Dr. med.Maurizio Calcagni and Prof. Dr. Pietro Giovanoli

Since high-energy shock waves in human beings revolutionized the treatment of urinary calculi, the application spectrum has successfully been widened to many other areas such as bony non-union, tendinosis and more recently chronic wounds and tissue ischemia. Prevention of ischemic flap necrosis or the treatment of acute and chronic wounds such as burns or decubital ulcers are just a few promising applications, which are supported by highly promising preliminary clinical data published in recent months.




Shock wave energy generated for medical purposes consists of a dominant pressure pulse which climbs steeply to some tenths or even hundreds of Mega-Pascals (MPa; 1 MPa = 10 bar) within several nanoseconds (nanosecond = 1/billionth of a second) and then falls again within several microseconds (microsecond = 1/millionth of a second); this wave is followed by a weaker tensile wave portion lasting for several microseconds.

Shock waves are characterized by transient pressure changes within a few nanoseconds reaching a maximum of about 100 MPa and then decreasing slightly less rapidly to reach a peak negative pressure level of approximately -10MPa. One of the most relevant indirect effects of shock waves is the cavitation phenomenon, which occurs during the second phase of the shockwave, when the negative pressure induces the formation of tiny vesicles. Through condensation of the fluid around them, which then diffuses into the vesicles, they may even increase in size. As soon as the pressure wave has passed, the vesicles collapse, causing a multidirectional asymmetrical fluid stream. Depending on the pulse rate of the shock wave this effect can cumulate therefore causing a powerful, yet tissue damaging jet stream. The physical importance of minor effects such as low-level induction of free radicals and transformation of a minimal part of the acoustic energy into very short-lived thermal energy is unclear. The aim of the here presented preliminary data was to assess the effect of shock wave energy on the microcirculation by the use of the mouse dorsal skinfold chamber to monitor microhemodynamics by intravital microscopy 1, 2, 6 and 24 hours after the impact. We found that functional capillary density was increased persistently during the 24 hours of observation, which was accompanied by a slight inflammatory response. Further studies are planned to characterize the relation between the dose, flux density and frequency of shock waves and the microcirculatory response. Our preliminary data suggest that shock wave energy may increase tissue oxygenation by recruiting “sleeping” capillaries.




Functional capillary density (A),rolling leukocytes (B),expressed as percentage of baseline (BL), in control animals (●) and animals treated with extracorporeal shock wave (derma PACE, E2 setting, 500 pulse, 8Hz) (○). Arrow indicates the treatment of shock wave. Note increase of functional capillary density and inflammation after shock wave treatment. Values are means±SD. *P< 0.05 vs. Control at corresponding time points.

## Achievements 2007

Talks

- Lindenblatt N. Endogene Hochregulation von eNOS verhindert endotheliale Dysfunktion und erhöhte mikrovaskuläre Thrombogenität unter Darbepoetin-α Behandlung. 124. Kongress der Deutschen Gesellschaft für Chirurgie (DGCh), München, 2007.
- Lindenblatt N. Upregulation of eNOS prevents endothelial dysfunction and increased microvascular thrombus formation during treatment with darbepoetin-alpha. 42nd Congress of the Society of Surgical Research (ESSR) Rotterdam, 2007.
- Lindenblatt N. Tacrolimus enhances microvascular thrombus formation in vivo most likely by increasing plasma levels of asymmetric dimethylarginine (ADMA). Chirurgische Forschungstage, Saarbrücken 2007.
- Lindenblatt N. Characterisation of the revascularisation of skin grafts in a new in vivo model– the role of angiogenesis. Chirurgische Forschungstage, Saarbrücken, 2007.
- Lindenblatt N. Darbepoetin-alpha führt bei intakter endothelialer Funktion nicht zur erhöhten mikrovaskulären Thrombogenität. 29. Jahrestagung der deutschsprachigen Arbeitsgemeinschaft für Mikrochirurgie (DAM), Zürich, 23.11.2007.
- Contaldo C. Die verbrannte Hand. 3. Workshop Verbrennungschirurgie, Zürich, 07.07.2007

Dissertation

- „Erythropoietin Protects Osteomyocutaneous Flaps from Reperfusion Injury: An Intravital Microscopy Study in Sprague Dawley Rats." work by Andrea Christina Schleh, accepted 2007 by the " Medizinische Fakultät Zürich".

Grants

- Research grant from the Helmut Horten Stiftung to Dr. med. C. Contaldo for the project "Nichterythroide Wirkungen von EPO in der plastisch-rekonstruktiven Chirurgie"
- European Board of Plastic and Reconstructive Surgery.
  Dr. med. C. Contaldo obtained his board certification in Madrid, November 2007.
- Best ECSAPS Paper Prize 2007(European Congress of Scientists and Plastic Surgeons) to Dr. Lindenblatt Nicole. Characterisation of the revascularisation of skin grafts in a new in vivo model– the role of angiogenesis.

## Collaborations:

- Prof. Dr. med. Michael D. Menger, Institut für Experimentelle Chirurgie, Universitätsklinikum, Homburg/ Saar, Deutschland
- Prof. Dr. med. Brigitte Vollmar, Institut für Experimentelle Chirurgie, Universität Rostock
- Prof. Dr. med. Otmar Trentz, PD Dr. Guido A. Wanner, PD Dr. Marius Keel, Dr. Christoph Meier, Klinik für Unfallchirurgie, Universitätsspital Zürich
- Dr. med. Jan Plock, Klinik für Plastische, Rekonstruktive und Aesthetische Chirurgie, Inselspital Bern
- Dr. med. Karl Lang, Institute of Experimental Immunology, Department of Pathology, University Hospital Zurich

## Selected references:


- Handschin AE, Jung FJ, Guggenheim M, Moser V, Wedler V, Contaldo C, Kuenzi W, Giovanoli P. Surgical treatment of high-voltage electrical injuries. Handchir Mikrochir Plast Chir 2007
- Zweifel CJ, Contaldo C, köhler C, Jandali A, Kuenzi W, Giovanoli P. Initial experiences using non-cultured autologous keratinocyte suspension for burn wound closure. J Plast Reconstr Aesthet Surg 2007
- Meier C, Contaldo C, Schramm R et al. Microdialysis of the rectus abdominis muscle for early detection of impending abdominal compartment syndrome. Intensive Care Med 2007
- El-Badry AM, Moritz W, Contaldo C, Tian Y, Graf R, Clavien PA. Prevention of reperfusion injury and microcirculatory failure in macrosteatotic mouse liver by omega-3 fatty acids. Hepatology 2007
- Erni D, Wetstein R, Contaldo C, et al. Hemoglobin vesicles to treat critical ischemia. Artificial Blood 2007.
- Pietsch AP, Lindeblatt N, Klar E. Perioperative hypothermia. Impact on wound healing. Anaesthesist. 2007
- Sorg H, Hoffmann JN, Rumbaut RE, Menger MD, Lindeblatt N, Vollmar B. Efficacy of antithrombin in the prevention of microvascular thrombosis during endotoxemia: an intravital microscopic study. Thromb Res. 2007
- Lindeblatt N, Platz U, Hameister J, Klar E, Menger MD, Vollmar B. Distinct effects of acute and chronic nicotine application on microvascular thrombus formation and endothelial function in male and female mice. Langenbecks Arch Surg. 2007
- Lindenblatt N, Menger MD, Klar E, Vollmar B. Darbepoetin-alpha does not promote microvascular thrombus formation in mice: role of eNOS-dependent protection through platelet and endothelial cell deactivation. Arterioscler Thromb Vasc Biol. 2007

## 2.4.3 Tissue Engineering

Dr. med.
Alexander E.
Handschin

PD Dr. med.
Guido Wanner

Dr. med.
Florian Jung

Dr. med.
Merlin Guggenheim

Sonja Hemmi

Manfred Welti

**Osteogenetic potential of adipose derived stem cells**

Dr. med. Alexander Handschin, PD Dr. med. Guido Wanner, Dr. med. Maurizio Calcagni, Prof. Dr. med. Pietro Giovanoli, Prof. Dr. med. Otmar Trentz

The repair of bone defects resulting from trauma is a common procedure in reconstructive surgery and may require the use of filling materials. Traditionally, surgeons have used autologeous bone grafting, e.g. from the iliac crest to treat such defects.The spongious bone marrow contains mesenchymal stem cells with a stem-cell-like character (bone marrow derived stem cells, BMSC). These cells have been shown to induce osteogenesis both in vivo and in vitro. However, access to bone marrow for autologeous bone grafting is associated with donor morbidity and insufficient tissue supply. Furthermore, autologeous bone grafting is limited in older patients due to a decline in BMSC numbers and differentiation potential with increasing patient age.

Similar to bone marrow, adipose tissue is developed from the mesodermal germ layer and contains a large, self-replenishing reservoir of stromal cells. The stromal fraction includes a heterogenous cell population including endothelial cells, smooth muscle cells, fibroblasts, mast cells and pre-adipocytes. In addition, similar to bone marrow, adipose tissue hosts a population of mutlipotent mesenchymal stem cells which are refered to as adipose derived stem cells (ADSC). ADSC show a multi-lineage potential with possible differentiation into the adipogenic, chondrogenic, myogenic and osteogenic cell line. The major advantage of fat tissue as a source for multipotent stem cells lies in the easy access and availablitiy, especially in Plastic and Reconstructive Surgery where large amounts of fat are rountinely harvested (Liposuction, Abdominoplasty, Mammareduction). Despite the recent enthusiasm for the use of ADSC in bone tissue engineering there is only limited research that has examined their behaviour and osteogenic potential. The aim of the current research in our laboratory is to evaluate the differentiation capacity of adipose derived stem cells using established in-vitro methods.

First, we want to test the influence of different culture mediums and external stimulation including growth-factors on the osteogenic potential of adipose derived stem cells. Another focus of the study is to test how long the ADSC are capable of maintaining the osteoblast phenotype in vitro. Finally, we are evaluating the role of extracellular matrix structure and composition on osteogenic differentiation using different 3D scaffolds and Integra ®, a dermal replacement layer made of a porous matrix of fibers of cross-linked bovine tendon collagen and a glycosamino-glycan (chondroitin-6-sulfate).

Only a few years ago, Zuk et al. first demonstrated the multilineage capacity of adipose-derived stem cells isolated from subcutanoues fat tissue. To date, differentiation of ADSC have been demonstrated into adipo-, osteo-, chondro-, myo-, cardiomyo-, endothelial-, hepato-, neuro-, epithelial- and haematopoiectic lineages (see Figure 1). When compared with bone marrow derived stem cells, ADSC possess further similarities in regards to growth kinetics, cell senescence, gene transcription, as well as CD surface marker expression. Taken together, the current research on osteogenetic potential of ADSC is focussing both on the development of optimal scaffolds and on the effect of osteoinducing factors in vitro. Although evidence to date suggests that ADSC may one day be useful in the treatment of difficult bone repairs, further investigations are required to determine their ultimate safety and efficiacy in a clinical setting.

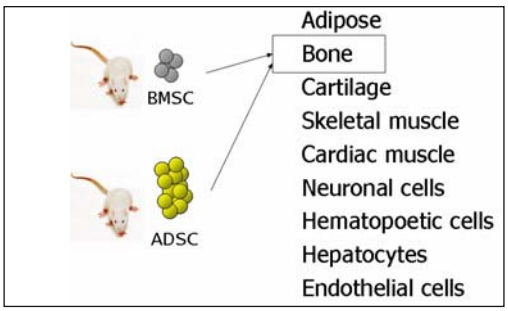



Figure 1:
Mesenchymal stem cells, traditionally obtained from bone marrow can also be isolated from fat tissue. Advantages of fat tissue as a source for human stem cell research include easy harvesting, availability, and low donor morbidity. Differentiation has been demonstrated for various tissue types.

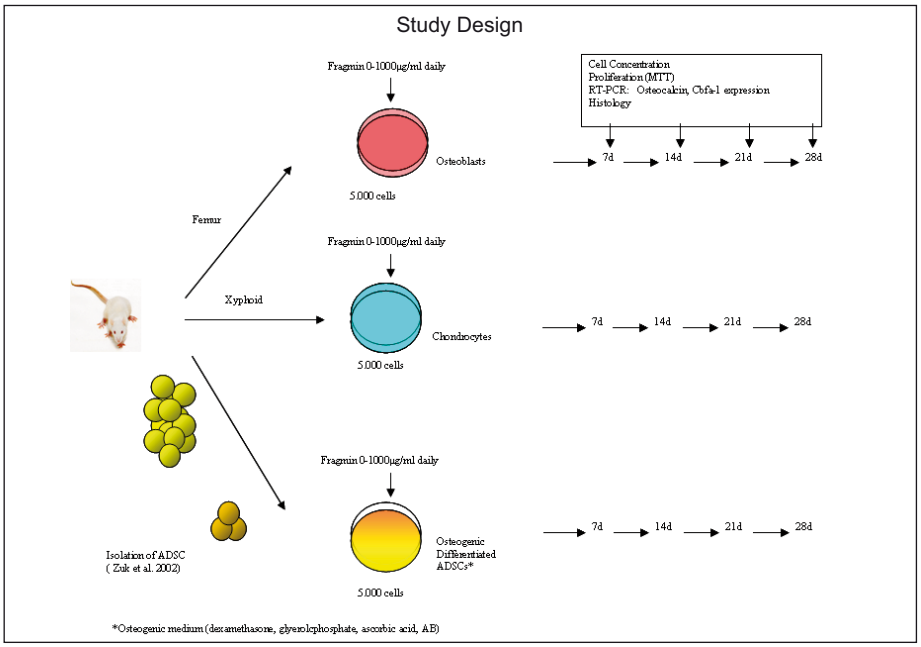
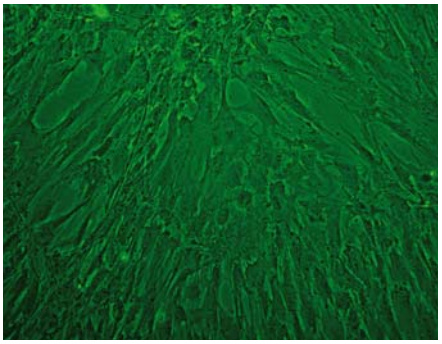
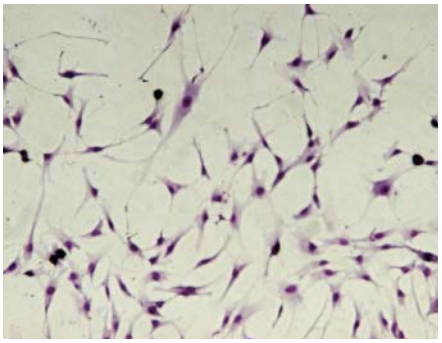



Figure 2:
Differentiation of ADSCs into several tissue types in vitro.



Adipose derived stem cells in vitro, Passage 1, 1:100



Osteoblasts in vitro, Passage 2, 1:100

Figure 3:
Osteojenic differentiated ADSCs in vitro (left) and primary osteoblast culture (right).

**Heparin inhibits proliferation of osteoblasts, chondrocytes and adipose-derived stem cells in vitro**

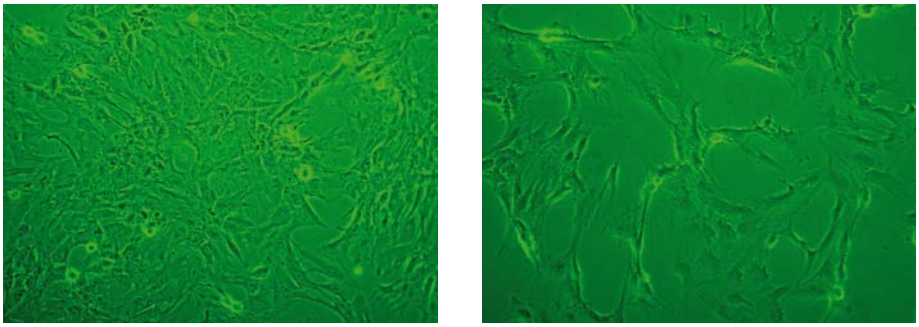
Dr. med. Alexander Handschin, Manfred Welti, Dr. med. Andrea Schleh, Dr. med. Maurizio Calcagni, Prof. Dr. med. Pietro Giovanoli, Prof. Dr. med. Otmar Trentz

Patients with long term heparin therapy may suffer from various side effects on different tissues, including thrombopenia, skin necrosis, and osteoporosis. Despite the introduction of new anticoagulants, heparin preparations are still considered a key element in thrombosis prophylaxis. The aim our present study was to further elucidate the interaction of heparin with various cell cultures from mesenchymal origin.

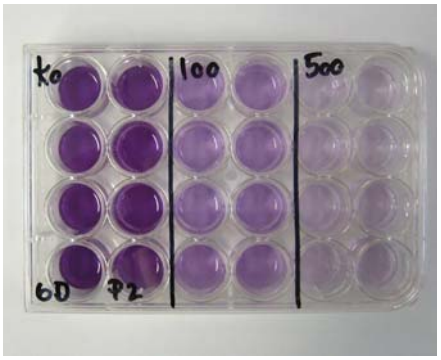
Three different cell culture systems where used for analysis. Osteoblasts where harvested from rat femur, and incubated at concentrations of 5.000 cells/well for a total of 4 weeks. Increasing doses of heparin (0-1000 µg/ml low molecular weight heparin, Fragmin ®,) were addeded daily. Chondrocyte cultures (rat xyphoid) and adipose-derived stem cells (perirenal fat, UCLA-protocol for isolation) were incubated at the same concentrations, heparin-dose and time. ADSCs were differentiated into the osteoblast lineage. Cell concentrations, proliferation rates (MTT-Test), protein synthesis (BioRad®) and phenotype expression (immunhistochemistry, osteocalcin, collagen) was measured at 7,14,21 and 28 days.

Osteoblast concentration and proliferation rates were significantly inhibited with heparin in a dose depending manner, resulting in a significant difference at high-dose treatment (MTT: day 7: -13.5% (500 µg/ml Fragmin); day 14: - 35.6%; day 21: - 45.2%; day 28: - 57.2% vs 100% control). Osteoblast phenotype expression (osteocalcin) was also strongly inhibited at high doses of heparin (>250 µg /ml). In comparison, chondrocyte proliferation rates were inhibited by heparin at an earlier point of time, and stronger extent ( -72% day 7, 100 µg/ml Fragmin). Proliferation of osteogenic differentiated ADSC was also inhibited by heparin at high doses (-30% day 7, -45% day 14, -48% day 21, -67% day 28, 500 µg/ml Fragmin vs control 100%)

The glycosaminoglycan heparin interacts not only with osteoblasts but also with cells from other tissues, including cartilage and fat. Heparin is a strong inhibitor of cellular proliferation and protein synthesis. Osteogenic differentiated ADSCs show a similar response to heparin incubation as mature osteoblasts, with inhibition of cellular proliferation at high-doses. This finding may be useful in regulating ADSC differentiation in vitro.



Left: ADSC (Rat) after 14days of culture of culture (osteogenetic medium, 1:100 Right: ADSC (Rat) after 14 days (osteogenetic medium + Fragmin 500µg/ml, 1:100). Note that under heparin incubation, cellular concentration is decreased and extracellular matrix mineralization is inhibited.




Proliferation of chondrocytes in vitro is inhibited by increasing doses of low molecular weight heparin (Fragmin ®). MTT Test. Control, 100µg/ml Fragmin, 500µg/ml Fragmin

## Achievements 2007

Vorträge und Kongressbeiträge 2007:

- Handschin AE, Trentz OA, Jung F, Wedler V, Wanner GA, Trentz O, Giovanoli P. Role of leptin on extracellular matrix mineralization of cells from heterotopic ossification.
  52nd Annual Meeting of the Plastic Surgery Research Council, Stanford University, USA, June 20nd-23rd 2007
  Plastic and Reconstructive Surgery 2007, 18 (Suppl 1): 74
- Handschin AE, Trentz OA, Jung F, Wedler V, Wanner GA, Trentz O, Giovanoli P. Ectopic bone formation in vitro – Role of leptin on extracellular matrix mineralization in vitro.
  EURAPS, Gent, May 18th-23rd 2007
- Handschin AE, Constantinescu M, Trentz OA, Vögelin E, Jung FJ, Wanner GA, Trentz O, Giovanoli P. Osteogenetic potential of adipocyte-derived stem cells compared with primary osteoblasts in vitro.
  14th IPRAS Congress, Berlin, June 26th-30th 2007.
- Handschin AE, Trentz OA, Jung F, Wedler V, Wanner GA, Trentz O, Giovanoli P. In-vitro effects of leptin incubation on cells from heterotopic ossification. 14th IPRAS Congress, Berlin, June 26th-30th 2007.
- Handschin AE, Bietry D, Hüsler R, Banic A, Constantinescu M. Surgical therapy of gynecomastia – A 10 year analyis.
  14th IPRAS Congress, Berlin, June 26th-30th 2007.
- Handschin AE, Jung FJ, Trentz O, Welti M, Schleh A, Contaldo C, Calcagni M, Giovanoli P. Heparin inhibits proliferation of osteoblasts, chondrocytes and adipose-derived stem cells in vitro.
  ECSAPS, Aachen, 21.-22.9.2007
- Handschin AE, Jung F, Künzi W, Giovanoli P. Surgical Treatment of High-Tension Electrical Burns. SGRPAC, Montreux 5.10.2007
- Jung FJ, Guggenheim M, Hug U, Handschin AE, Künzi W, Giovanoli P Von der Zelle zur Haut – Erfahrungen mit autologen Keratinozyten-Sheets SGRPAC, Montreux 5.10.2007

Sonstige Vorträge 2007

- Handschin AE. Wundbehandlung und Wundprotektion bei Verbrennungen. 3. Workshop Verbrennungschirurgie, Klinik für Wiederherstellungschirurgie USZ 7. Juli 2007
- Handschin AE. Gynäkomastie. Hausarztfortbildung Brustchirurgie. Klinik für Wiederherstellungschirurgie 13. Dezember 2007

**Collaborations:**

- Frau Dr. O. A. Trentz, Institute of Research MIOT Hospital Chennai, India
- Division of Trauma 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

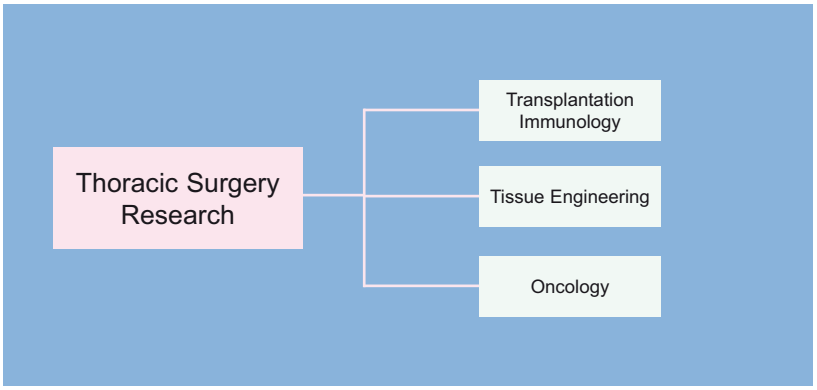
**Selected references:**


- Handschin AE, Trentz OA, Hemmi S, Wedler V, Giovanoli P, Trentz O, Wanner GA. Leptin increases extracellular matrix mineralization of osteoblasts from heterotopic ossification and normal bone. Ann Plast Surg, 2007, 59: 329-333
- Handschin AE, Jung FJ, Guggenheim M, Wedler V, Contaldo C, Moser V, Künzi W, Giovanoli P. Surgical Treatment of High-Voltage Electrical Injuries. Handchir Mikrochir Plast Chir 2007, 39: 345-349
- Handschin AE, Banic A, Constantinescu M. Pulmonary embolism following plastic surgery tourism. Clin Appl Thromb Hemost, 2007, 13: 340
- Handschin AE, Cardell M, Contaldo C, Trentz O, Wanner GA. Functional results of angular-stable plate fixation in proximal humeral fractures. Injury accepted October 7th 2007, in press
- Kock HJ, Huber FX, Handschin AE, Meeder PJ. In-vitro studies on various PMMA Bone cements. Z Orthop Unfall accepted Dec 12th 2007, in press

## 2.5 Thoracic Surgery Research

PD Dr. med.
Sven Hillinger

Prof. Dr. med.
Walter Weder




### 2.5.1 Transplantation Immunology

PD Dr. med.
Stephan Korom

PD Dr. med.
Sven Hillinger

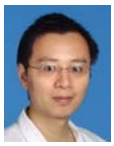
Lung transplantation has become an effective therapeutic option in the treatment of patients with end-stage pulmonary diseases. However, early acute graft dysfunction continues to be a serious obstacle to successful lung transplantation, accounting for significant postoperative morbidity and mortality. In our established large and small animal models of unilateral lung transplantation we investigated different substances in terms of early graft function improvement. Wolfgang Jungraithmayr, who joined our group this year, put an enormous effort in this field, especially the problem of chronic rejection. He successfully performed the first series of orthotopic unilateral lung transplantations in mice, which will open the field of lung transplantation research in many directions.

PD Dr. med.
Ilhan Inci

Dr. med.
Markus Cardell

**The effect of organ-specific CD26/dipeptidylpeptidase IV (DPP IV)-inhibitor – preconditioning on acute pulmonary allograft rejection in rats**
W. Jungraithmayr, B. Oberreiter, M. Cardell, W. Zhai, S. Hillinger, S. Arni, S. Korom

Dr. med.
Wolfgang
Jungraithmayr



Dr. med.
Wei Zhai

The T cell activation Ag CD26/DPP IV combines costimulatory and enzymatic properties. Its catalytic epitope functions as dipeptidyl peptidase IV (DPP IV), an exopeptidase that modulates the biological activity of chemokines/interleukins by dipeptide cleavage. Targeting systemic enzymatic activity has abrogated acute and accelerated rejection in rat heart and lung transplantation (Tx), and local perfusion of pulmonary grafts has reduced ischemia/reperfusion injury. We investigated the combined graft-preserving- and anti-rejection-effect of pulmonary inhibitor preconditioning on the early post-Tx phase.
An allogeneic rat orthotopic single lung Tx model was employed (LBNF1 to LEW). Control lungs (I) were flushed with Perfadex® vs. experimental grafts (II), perfused with Perfadex®+AB192 (diphenyl phosphonate, small mol. weight inhbitor) (25µmol/L) and transplanted (ischemia ≤ 45 min). Both groups were treated with cyclosporin (CsA) at a dose of 2.5 mg/kg/day. Analysis on day 5 included $pO_2$, $pCO_2$ and histological acute rejection (AR) grading (ISHLT).

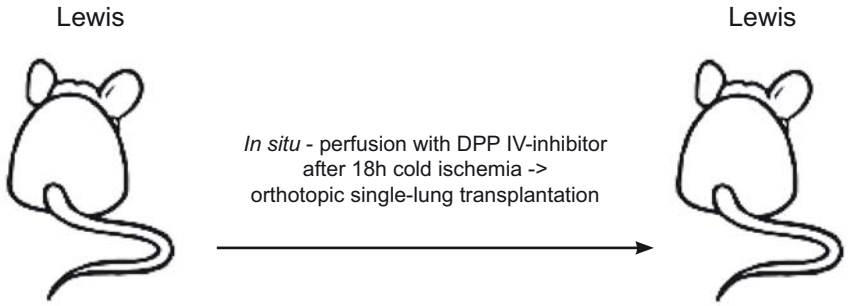
Dr. rer. nat.
Stephan Arni

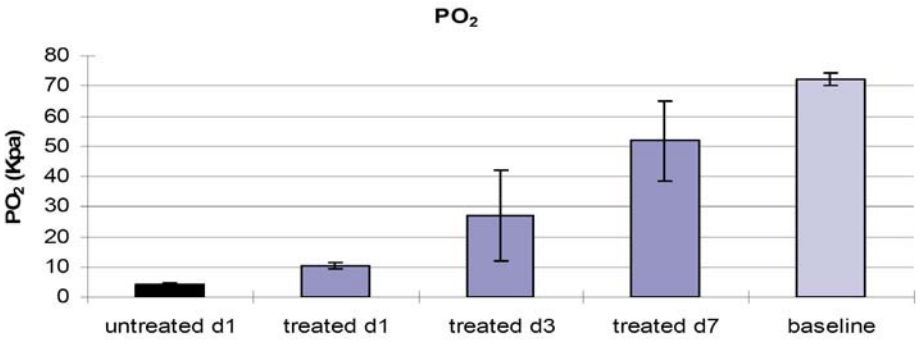
Dr. med.
Brigit Oberreiter

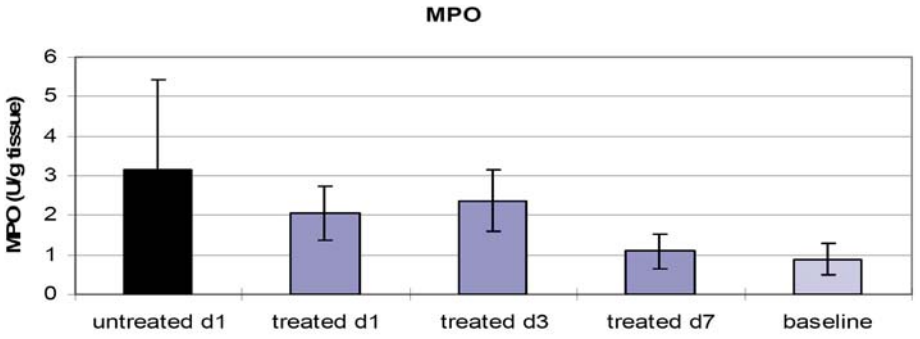
At day 5 post Tx, pulmonary grafts preconditioned with AB192 showed an preserved oxygenation capacity (II: 109±33mmHg vs I: 53±19mmHg) with stable $pCO_2$ (II: 56±15mmHg vs I: 58±3 mmHg). Histologically, group-II-grafts revealed minimal to mild AR (ISHLT-Grading A: 1.6±0.5), whereas group I showed moderate/severe AR (ISHLT-Grading A: 3.6±0.6) with evidence of bronchiolar inflammation and massive infiltration of macrophages and lymphocytes.
In spite of the non-marginal character of the grafts and immunosuppressive therapy, organ-specific enzymatic inhibition further strikingly attenuated transplant-associated immunogenicity, leading to the abrogation of AR. Further studies are needed to decipher the complex interplay of enzyme- and immune-functions associated with CD26/DPP IV.

**Delayed pulmonary graft function: The role of CD26/DPP IV**
W. Jungraithmayr, W. Zhai, M. Cardell, S. Hillinger, S. Arni, S. Korom

Systemic inhibition of CD26/DPP IV enzymatic activity has abrogated graft rejection in rat cardiac and pulmonary transplantation models. Organ-specific catalytic inhibition of lung explants prior to implantation markedly decreased ischemia/reperfusion injury and preserved pulmonary function at 2 hours post-perfusion. Here we investigate the influence of organ-specific inhibitor-preconditioning on the long-term course of delayed pulmonary graft function due to extended ischemia.
A syngeneic rat (LEW) orthotopic left lung transplantation model was employed (n=5-6/group). As inhibitor served AB192 (bis(4-acetamidophenyl) 1-(S)-prolylpyrrolidine-2(R,S)-phosphonate). Donor lungs in group I and II (controls) were flushed and preserved in Perfadex® for 18h at 4ºC, then transplanted and harvested after 24-h (II) or 7d (I). Group IV, V and VI grafts were perfused/stored in Perfadex®+25µmol/L AB192 for 18h at 4ºC, and harvested at 24-h (IV), d3 (V) and d7 (VI). Lungs in group III were treated as controls (I, II), but received in addition the antioxidant melatonin, harvested after 7d. Primary endpoint was survival. Secondary endpoints (at harvest), included blood gas analysis, peak airway pressure (PAwP), wet/dry (W/D) weight ratio, myeloperoxidase activity (MPO) and thiobarbituric acid reactive substances (TBARS).
Survival was significantly better between groups VI (80%) vs. III (40%) and I (16.3%) ($p<0.01$) at 7d. At 24h, pulmonary function was significantly superior in group-IV- vs. group-II-grafts: $pO_2$ was 78.7±7.1 vs. 29.8±5.7mmHg ($p<0.01$); PAwP was 19.3±1.1 vs. 24.3±2.9mmHg ($p<0.01$); W/D ratio was 6.7±1.3 vs. 9.5±1.5 ($p<0.05$); TBARS was 1.1±0.6 vs. 2.5±0.7µM ($p<0.05$). AB192-preconditioned grafts (IV, V, VI) continuously improved following implantation, reaching near-baseline measurements at the d7-timepoint.
Pulmonary perfusion with a novel specific inhibitor (AB192) of CD26/DPP IV enzymatic activity significantly reduces extent and mortality of delayed lung graft function and accelerates recovery after extended ischemia.




| Gruppe (nach 24h) | I | III | p-wert |
|---|---|---|---|
| pO2 (mmHg) | 78,7±7 | 29,8±5,7 | p<0,01 |
| PAwP (mmHg) | 24,3±2,9 | 19,3±1 | p<0,01 |
| Dry/wet-ratio | 9,5±1,5 | 6,7±1,3 | p<0,05 |
| MPO (µM) | 2,5±0,7 | 1,1±0,6 | p<0,05 |

**Chronic Rejection in Sensitized Lung Allograft Recipients**

W. Jungraithmayr, S. Arni, S. Korom

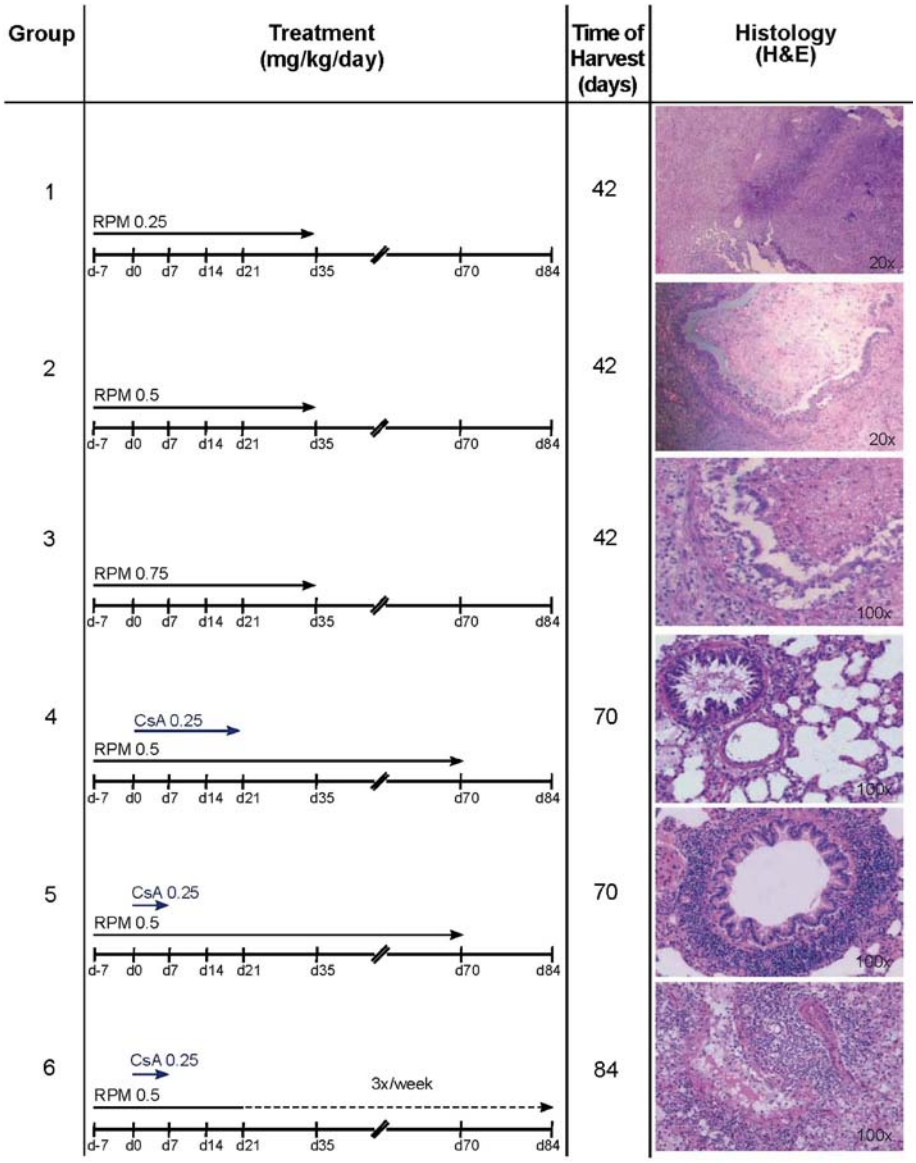
Sensitization of transplant recipients toward major histocompatibility antigens (MHC-Ag) remains a challenge in modern transplantation medicine. These patients experience an increased rate of acute rejection episodes compared to unsensitized individuals, which - even successfully treated - predispose for a progressive failure of graft function (chronic rejection, CR) over time. In spite of remarkable success in prevention and treatment of acute rejection, modern immunosuppressive therapy has not substantially affected the incidence or course of CR. Multiple etiologic factors, both immunologic and non-immunologic, are contributing to the development of CR.

Within 3 years post-transplantation, in more than 60% of all pulmonary grafts, fibro-obliterative remodeling of the small airways has been found on transbronchial biopsy, correlating with a progressive and most often irreversible decline in ventilatory function. The pathological hallmark of CR in lung transplants is bronchiolitis obliterans (BO) triggered by lymphocytic infiltration of bronchiolar structures, which induces a fibroproliferative response, eventually leading to luminal obstruction of medium and small bronchioli. Once BO is emerging, modification of immunosuppressive therapy may only ameliorate its dynamics, but will not reverse the impeding deterioration of graft function. In spite of the impact of chronic pulmonary rejection on patient morbidity and mortality, straightforward experimental models addressing CR in transplanted lungs do not exist. We propose a clinically relevant and reproducible model of CR based on sensitized recipients receiving unilateral orthotopic lung transplants.

Based on experience in establishing a cardiac CR model, and advancing our studies in pulmonary allograft transplantation in sensitized rat, we want to develop a reproducible experimental setup to study BO. In a first proof-of-concept approach, we have identified treatment regimen and timeframe to initiate BO-like changes in orthotopic pulmonary grafts in sensitized rat recipients.

LEW-rats, sensitized with BN-skin, receive left lung transplants from $LBNF_1$-donors, seven days later. The development of CR is modulated by a sequential treatment pattern with rapamycin (RPM) and cyclosporine (CsA). Ventilatory function tests, intragraft assay of Th1/Th2 cytokine mRNA and protein expression (RT-PCR, immunohistochemistry) are used to track and document the course of BO.

We propose a relevant and reproducible approach for studying CR in lung transplantation. This model will allow for better understanding of the pathological changes at the level of small airways during the course of BO. Subsequently, it will serve as a standardized in vivo setup and workbench for testing of future therapeutic regimen in treating chronic pulmonary rejection.




Approach to the development of a chronic rejection model in rat lung allograft recipients

**Ex-vivo reconditioning of marginal donor lungs injured by acid aspiration**

I. Inci, B. Leskosek, S. Arni

Injured lungs due to gastric acid aspiration may be rejected for transplantation because of the possibility of early graft dysfunction. Several experiments have demonstrated that the pulmonary surfactant system is harmed in acute lung injury. We hypothesized diluted surfactant administration during ex vivo perfusion would recondition the lungs injured by acid aspiration and permit their use as suitable grafts for transplantation.

Lung injury was induced by 5 ml/kg with betaine-HCl/pepsin mixture (pH: 1.5) via flexible bronchoscope. After injury pigs were randomly assigned into 3 study groups (n=6); saline lavage during exvivo perfusion (Control); surfactant lavage exvivo (SL-Exvivo); surfactant lavage before harvest (SL-Pre). Normal group (n=4); no lung injury. Cold storage time was 3 hours. A volume of 10 ml/kg (4 mg/ml, 40 mg/kg) surfactant (Curosurf®) was used for lavage. Bronchoalveolar lavage (BAL) was performed before and after injury and at the end of the experiment. Surfactant function, phospholipids, protein and neutrophil percentage in BAL were assessed. Hemodynamic and aerodynamic parameters were measured every 30 min during 2 hours of observation period. At the end of observation period wet to dry weight ratio was calculated and histological examination was performed.

Approximately 50% decrease in $PaO_2$ was observed in all animals after injury. Ex vivo surfactant lavage resulted in lower pulmonary vascular resistance, lower oxygenation index and higher $PaO_2/FiO_2$ ratio compared to control group ($p=0.001$, $p=0.0001$, and $p=0.0001$, respectively. Analysis of variance for repeated measures, Fig 1, Fig 2). Wet to dry weight ratio was lower in SL-Exvivo compared to control group ($p=0.015$, Fig 3). BAL neutrophil percentage at the end of the experiment (BAL3) significantly differed among the groups (Kruskal-Wallis test $p=0.001$). Control versus all groups $p<0.05$, SL-Exvivo versus SL-Pre $p=0.04$ (Fig 4). BAL cytokine levels: There were no statistical differences among the groups regarding cytokine levels in BAL. However, the trend was lower in surfactant treated groups compared to control group (Figures 5, 6). BAL protein levels were lower in SL-Exvivo group compared to HCL-Control group ($p<0.05$, Fig 7).

We conclude that diluted surfactant lavage during ex-vivo perfusion improves the graft function of lungs injured by gastric aspiration.

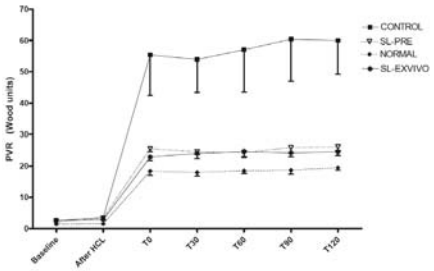



Figure 1: Pulmonary vascular resistance

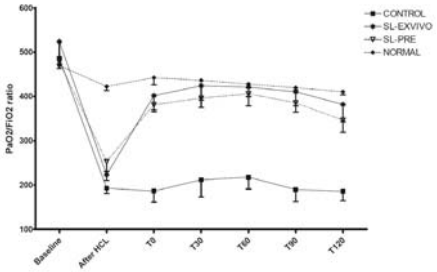



Figure 2: PaO2/FiO2 ratio

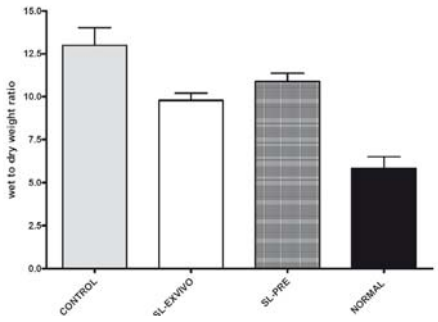



Figure 3: Wet to dry weight ratio

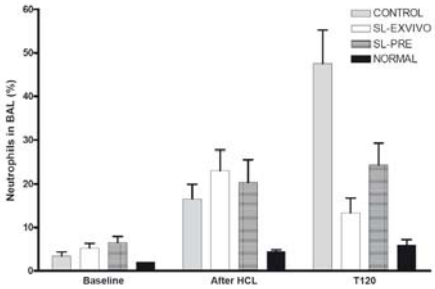



Figure 4: Neutrophil % in BAL

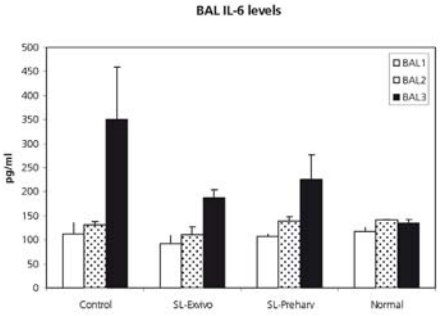



Figure 5: BAL IL-6 levels

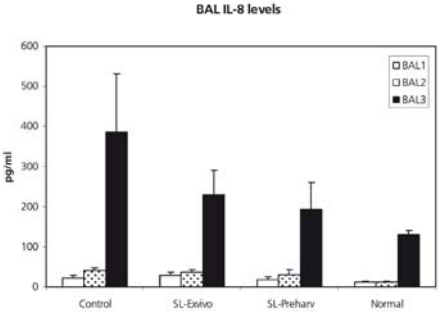



Figure 6: BAL IL-8 levels

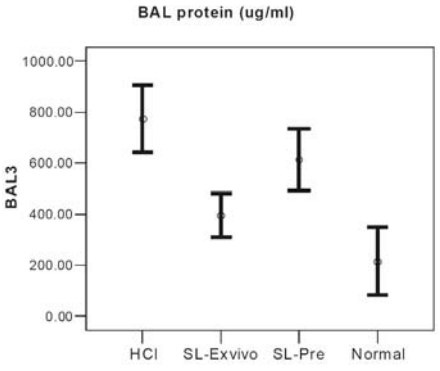



Figure 7: BAL protein levels

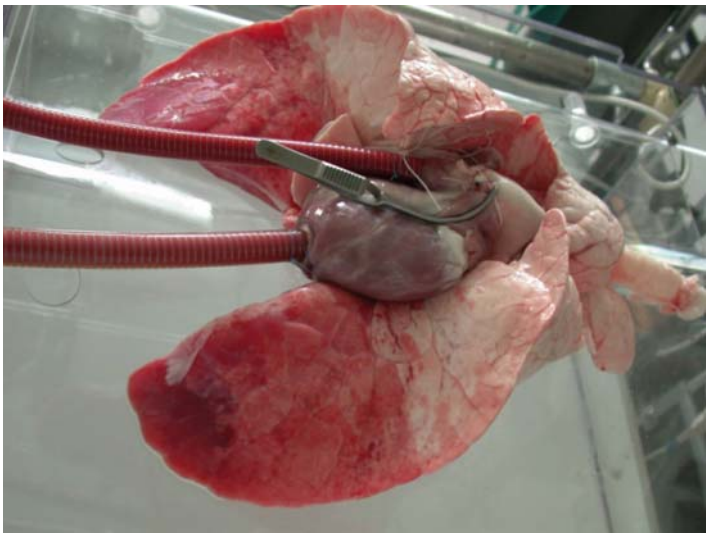


Figure Ex-vivo perfusion of the lung graft

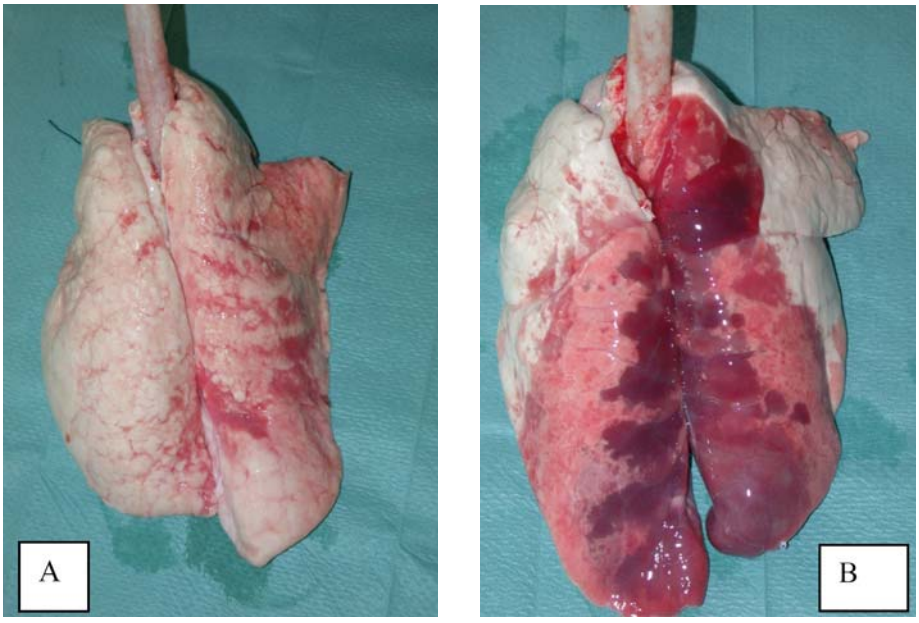



Fig. A: Normal Lung (without gastric acid aspiration) and B: after aspiration

## Achievements 2007

- DFG grant (DFG - JU 2730/1-1), March 2007
- EMDO Stiftung, September 2007
- Establishment of a mouse model of orthotopic, unilateral lung transplantation

Presentations (I. Inci):

- Society of Thoracic Surgeons Annual Meeting, San Diego, USA. 2007: N-acetylcysteine attenuates lung ischemia-reperfusion injury after lung transplantation
- The International Society for Heart and Lung Transplantation Annual Meeting 2007, San Francisco, USA. Fibrinolytic treatment improves the quality of lungs retrieved from non-heart-beating donors.

## Collaborations:

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

## Selected references:


- Korom S, De Meester I, Stadlbauer TH, Chandraker A, Schaub M, Saygeh MH, Belyaev A, Haemers A, Scharpe S, Kupiec-Weglinski JW. Inhibition of CD26/dipeptidyl peptidase IV activity in vivo prolongs cardiac allograft survival in rat recipients.Transplantation. 1997 May 27;63(10): 1495-1500.
- Jung FJ, Yang L, De Meester I, Augustyns K, Cardell M, Hillinger S, Vogt P, Lardionis D, Scharpe S, Weder W, Korom S. CD26/dipeptidylpeptidase IV-targeted therapy of acute lung rejection in rats.J Heart Lung Transplant. 2006 Sep;25(9):1109-1116.
- Zhai W, Cardell M, De Meester I, Augustyns K, Hillinger S, Inci I, Arni S, Jungraithmayr W, Scharpe S, Weder W, Korom S. Intragraft DPP IV inhibition attenuates post-transplant pulmonary ischemia/reperfusion injury after extended ischemia. J Heart Lung Transplant. 2007 Feb;26(2):174-180.
- Inci I, Zhai W, Arni S, Inci D, Hillinger S, Lardinois D, Vogt P and Weder W. Fibrinolytic treatment improves the quality of lungs retrieved from non-heart-beating donors. J Heart Lung Transplant. 2007;26:1054-60.
- Inci I, Zhai W, Arni S, Hillinger S, Vogt P, Weder W.N-acetylcysteine attenuates lung ischemia-reperfusion injury after lung transplantation. Ann Thorac Surg. 2007 Jul;84(1):240-6.

## 2.5.2 Tissue Engineering

Dr. med.
Qiang Tan

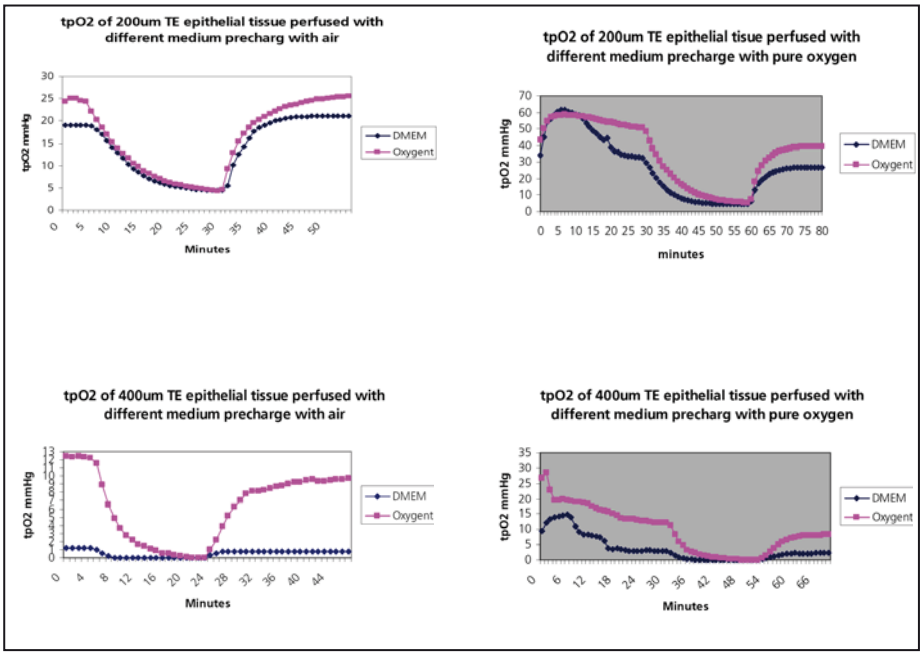
Manfred Welti

**Effect of artificial oxygen carrier on tissue engineered trachea reconstruction**

Q. Tan, M. Welti

As part of our tissue engineering project for a novel trachea construct we tested the effect of artificial oxygen carrier (OxygentTM) on epithelia metabolism, cartilage tissue formation, angiogenesis process and epithelial tissue oxygen pressure tPO2 level during tissue engineered trachea reconstruction.
Medium supplemented without 10% volume of OxygentTM was compared with normal medium in all assessments. Microdialysis is employed to monitor epithelial metabolism cultured with two kinds of medium. Chondrocyte-scaffold construct were cultured with different medium for one month before glycoaminoglycan (GAG) and histology assessments. Xenogenic (porcine) acellular dermal matrixes (ADM) immersed in mediums were put on the top of chick embryo chorioallantoic membrane (CAM) model. The vessel density around the scaffolds were compared by orthogonal polarization spectral OPS after five days. The tpO2 was measured with polarographic microprobes at different thickness inside tissue engineered tracheal epithelium perfused with mediums underneath.
Cultured with OxygentTM supplemented medium, epithelia showed less lactate (0.633±0.0814 v.s 0.8±0.063 mmol/l, $\rho<0.05$) and lactate/pyruvate L/P ratio (1.776±0.330 v.s 3.046±3.88, $\rho<0.01$) while the glucose concentration remains similar (5.545±6.21 v.s 6.131±1.27, $\rho>0.05$). After long-term culture with OxygentTM medium, the chondrocyte-scaffold construct showed less GAG value (0.029±0.004 v.s 0.132±0.0008, $\rho<0.05$) and the histology results also demonstrated poor extra-cellular cartilage tissue matrix formation.
Regarding angiogenesis, statistic analysis found no difference of vessel density around the ADM preconditioned with different mediums in CAM model. The polarographic microprobe proved significant increase of epithelial tpO2 with OxygentTM medium perfusion, 51.22±0.28 v.s 33.35±0.26 mmHg at 200um thickness and 12.48±0.13 v.s 3.08±0.07mmHg at 400um, $\rho<0.01$.
Continuous medium perfusion mimics normal blood flow and artificial oxygen carrier which functions as hemoglobin significantly increase the tissue engineered trachea epithelial tpO2. The deposite of OxygentTM inside the scaffold benefits the epithelium metabolism, shows no side effect on angiogenesis while hampers the formation of cartilage tissue.

Tissue engineered tracheal epithelial tissue tpO2 measurement




The polarographic microprobe was first checked by measuring the pO2 level of the air inside the incubator (131.32 ±2.53mmHg). The epithelium tPO2 measurement results were summarized in Figs with two curves in each picture represent the changes of tpO2 under perfusion of DMEM and Oxygent DMEM respectively. Each curve can be separated into three parts: Phase I reflect the situation under continuous mediums perfusion; Phase II represents the decrease of tPO2 after we stop the perfusion; Phase III represents the resume of tPO2 after we restart the perfusion.

The data showed, under continuous perfusion of DMEM reoxygenated with air, the tpO2 level is 18.97±0.03 mmHg in the proximal TE epithelial tissue and 1.17±0.03mmHg distally. The corresponding data in the situation of Oxygent DMEM perfusion are 24.66±0.14 mmHg and 12.23±0.14mmHg respectively and both showed significant differences compared with DMEM group data ($\rho<0.01$). After we stop the pump, the tpO2 level declined precipitously to 4.67±0.07mmHg in the proximal part and 0 mmHg in the distal part. The level resume at a rate of 1.51mmHg/min proximally; 0.08mmHg/min distally in the DMEM group and 1.73mmHg/min; 0.83mmHg/min respectively in the Oxygent DMEM group. By compared the area under phase II, we conclude 10% more oxygen content of the Oxygent DMEM in the proximal part of the TE epithelial tissue and 45times more distally.

With the perfusate reoxygenated with pure oxygen, the tpO2 increase to 33.35±0.26 mmHg proximally and 3.08±0.07mmHg distally under continuous perfusion of DMEM. The tpO2 dropped to 5.5±0.1mmHg and 0mmHg respectively within 15minutes after we stopped the perfusion pump. The level resume to 27±0mmHg (at rate of 2.09mmHg/min) proximally and 2.38±0.06 (at rate of 0.21mmHg/min) distally within the same time phase when we restarted it. On the situation of Oxygent DMEM, the tpO2 level is 51.22±0.28mmHg proximally and 12.48±0.13mmHg distally under continuous perfusion; dropped precipitously to 5.5±0.1mmHg and 0mmHg respectively within 30minutes. The levels resume to 39.3±0mmHg proximally and 8.08±0.04mmHg distally within 15minutes. There are significant differences regarding the tpO2 level in both proximal and distal parts of the TE epithelial tissue between DMEM and Oxygent DMEM groups. By measure the area under Phase II, we compared the oxygen content in the Oxygent DMEM to be 33% more than that in DMEM in the proximal part and 300% more distally.

## Achievements 2007

- Swiss National Foundation (SNF) project no. 116807

## Collaborations:

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

## 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.
- Tan Q, Steiner R, Yang L, Welti M, Neuenschwander P, Hillinger S, and Weder W. Accelerated angiogenesis by continuous medium flow with vascular endothelial growth factor inside tissue-engineered trachea. Eur J Cardiothorac Surg. 200731(5), 806.

## 2.5.3 Oncology


PD Dr. med.
Sven Hillinger


Dr. med.
Stephan Arni


Dr. med.
Isabelle Opitz


PD Dr. med.
Didier Lardinois


Dr. med.
L. Ampollini


Dr. med.
Markus Cardell


Manfred Welti

**Immunotherapy for lung cancer**
S. Hillinger, S. Arni

We previously reported an efficient treatment based on the injection of both the commercially available chemokine CCL19 and cytokine IL7 to eradicate lung tumors in murine models. In our ongoing SNF proposal “Immunotherapy for lung cancer” we will produce murine myeloid dendritic cells (mDC) expressing both the chemokine CCL19 and IL7 via adenoviruses. Our final goal is to inject therapeutically transformed mDC in tumour bearing mice. During 2007 we 1) established the mDC culture, 2) produced CCL19 and IL7 and control adenoviruses.

**1) Establishement of murine myeloid dendritic cell (mDC) culture and phenotypical characterisation after TNF alpha or LPS maturation.**
We followed the protocol of Lutz et al [1] where the mDC cells are isolated from femurs of C57BL/6 female mice. Long term culture (up to 12 days) increased the risk of contamination without improving the yields of mDC. After 8 days of expansion in GMCSF conditioned media [2] we stimulated cells with TNF alpha and LPS, two reageants known to induce differenciation/maturation. As already described [3] we also observed that TNF alpha or LPS (shown in figure 1B) are potent *in vitro* maturation signal for mDC.

Figure 1A

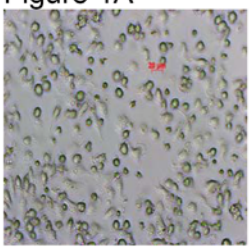


Figure 1B

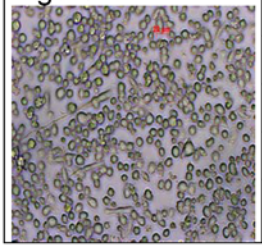


**Table 1**

| | Antibody FL-1 | Antibody FL-2 | % of DP |
|---|---|---|---|
| mDC + TNFa | Anti CD80 FITC | Anti Class 2 PE | 53.6 |
| mDC + LPS | Anti CD80 FITC | Anti Class 2 PE | **75.3** |
| mDC + PBS | Anti CD80 FITC | Anti Class 2 PE | 32.1 |
| mDC + TNFa | Anti CD86 FITC | Anti Class 2 PE | 49 |
| mDC + LPS | Anti CD86 FITC | Anti Class 2 PE | **76.8** |
| mDC + PBS | Anti CD86 FITC | Anti Class 2 PE | 31.8 |
| mDC 1 + TNFa | Anti CD11c FITC | Anti Class 2 PE | 54.5 |
| mDC + LPS | Anti CD11c FITC | Anti Class 2 PE | **77.6** |
| mDC + PBS | Anti CD11c FITC | Anti Class 2 PE | 30.9 |

Murine dendritic cells (mDC) were grown for 8 days in conditioned media and maturated with either LPS at 1 ug/ml for 24 hours as in Figure 1B or left unstimulated as in Figure 1A . In Table 1 mDC were then maturated with either TNFa 500U/ml or 1 ug/ml of LPS or PBS and after appropriate double antibody staining the R1 mDC gated population was analysed by FACS. The % of R1 DP cells are the result from one representative experiment.

Voigtlander et al [3] presented evidences for difference in the restimulating capacities of either LPS or TNF alpha matured mDC. In Table 1, we characterized by FACS phenotypical markers for mDC maturation [1]. After 8 days in culture mDC were maturated either with TNF alpha at 500 U/ml or with LPS at 1 ug/ml or PBS. Double cell surface immunostaining were performed with monoclonal anti mouse FITC CD80 or FITC CD86 or FITC CD11c in combination with PE labelled Class 2 and isotype control antibodies. We repeatedly observed that mature CD11c/Class 2 double positive (DP) mDC were more abundant after LPS maturation (77.6 % DP) than with TNF alpha (54.5 % DP) or unstimulated mDC (30.9% DP).
We conclude that a good yield of mDC with a short term culture of 8 days versus 12 days as described [1] is achievable in our hand. Moreover, either LPS at 1 ug/ml or TNF alpha at 500 U /ml for 24h are efficient maturating signals. Since component of adenoviruses may also stimulate mDC (as LPS on Toll like receptor 4) we will need further in vitro experiment to phenotypically characterise adenovirus infected mDC.

**2) Preparation of the AdmCCL19 AdmIL7 and the control AdWT adenoviruses**

We obtained pAdHM15RGD and pAdHM15RGD-CCL19, two plasmids encoding for replication incompetent type 5 adenovirus from the group of Dr. S. Nagakawa [4, 5]. Wild type type 5 adenoviruses binds to the CAR (Cocksackie B and adenovirus receptor). Both of our plasmids contain an additional RGD coding sequence. RGD fiber-mutant adenoviruses are then also binding the alpha5/beta5 and alpha5/beta3 integrins involved in a second step of adenoviruses internalisation. Conventional adenovirus vector do achieve very poor gene transduction to mDCs but RGD fiber-mutant appears to be very successful to achieve this goal [6].
In both plasmids restriction analysis for the *Pac-I* (2 RE sites) and *Bgl-II* (8 RE sites) were selected and analysed (see Figure 2). Typically and for both plasmids, as shown in figure 2 lane 3, the *Pac-I* restriction enzyme digestion give a 3 kb band whereas the 30 kb bands correspond to the virus genome co-migrating with the 23 kb *Hind III* lambda phage digest. We selected several of those positive clones (see Figure 2 lane 3 and also less clearly lane 9).

Figure 2

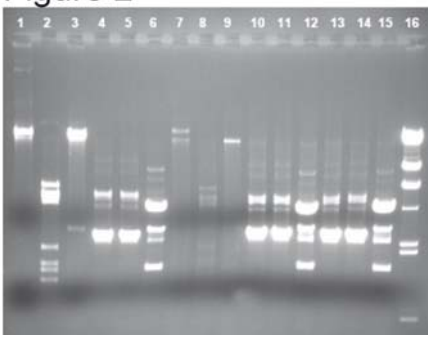



Figure 2
Restriction enzyme analysis pattern loaded on 0.7% agarose gel showing 2 different preparations of pAdHM15RGD (lanes 1 to 6) and 3 other preparations of pAdHM15RGD-CCL19 (lanes 7 to 15)
Lane 1, 4, 7, 10, 13 are uncut 33 kDa plasmids. Lanes 2, 5, 8, 11, 14 are Bgl-II digests ( 8 cuts) and lanes 3,6,9,12,15 are Pac-I digests (2 cuts) Lane 16 are lambda HindIII digest (approx 23,1; 9, 4; 6,5;4,3; 2,3; 2; 0.5 kB)

Selected and amplified clones were DNA sequenced and we obtained a 100% match with sequence NM_011888 corresponding to the *Mus musculus* chemokine (C-C motif) ligand 19 (Ccl19) mRNA. We transfected AD-293 cells (Stratagene) and then amplified several time in HEK293 cells to obtain high title stock.
For mouse IL-7 cytokine adenovirus we obtained both a viral stock of mIL7 and control from Dr S. Sharma. All the viral stock obtained were quantitated through the end point dilution method and are in the range of 10e+8 to 10e+7 pfu/ml.
We will perform bioassays to measure the biological activity of the molecules produced in the culture supernatant (either survival of IL-7 dependant 2E8 cells or chemotaxis of immune cells via CCL19).

## Achievements 2007

- Presentation at the Annual Meeting of the AACR, Los Angeles, CA, April 2007

## Collaborations:

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

## Selected references:


- Lutz, M.B., et al., An advanced culture method for generating large quantities of highly pure dendritic cells from mouse bone marrow. J Immunol Methods, 1999. 223(1): p. 77-92.
- Zal, T., A. Volkmann, and B. Stockinger, Mechanisms of tolerance induction in major histocompatibility complex class II-restricted T cells specific for a blood-borne self-antigen. J Exp Med, 1994. 180(6): p. 2089-99.
- Voigtlander, C., et al., Dendritic cells matured with TNF can be further activated in vitro and after subcutaneous injection in vivo which converts their tolerogenicity into immunogenicity. J Immunother, 2006. 29(4): p. 407-15.
- Gao, J.Q., et al., Anti-tumor responses induced by chemokine CCL19 transfected into an ovarian carcinoma model via fiber-mutant adenovirus vector. Biol Pharm Bull, 2005. 28(6): p. 1066-70.
- Okada, N., et al., Tumor suppressive efficacy through augmentation of tumor-infiltrating immune cells by intratumoral injection of chemokine-expressing adeno-viral vector. Cancer Gene Ther, 2005.
- Hillinger S, Yang SC, Zhu L, Huang M, Duckett R, Atianzar K, Batra RK, Strieter RM, Dubinett SM, Sharma S. Epstein Barr Virus-Induced Molecule 1 Ligand Chemokine (ELC/CCL19) Promotes IFNg-dependent Antitumor Responses in a Lung Cancer Model. J Immunol. Dec 15; 171(12):6457-65 (2003)
- 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 Bronchoal-veolar Cell Carcinoma. Hum Gene Ther. Nov;14:1511-24 (2003)

**Malignant pleural mesothelioma –intrapleural therapy after surgery**

I. Opitz, L. Ampollini, S. Arni, D.Lardinois

**Background:** Malignant pleural mesothelioma is an aggressive tumour with increasing incidence that is expected to peak in the next two decades. The man-agement 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 tumour control with promising results in early clinical and experimental studies but further refinement is still necessary. Beside chemotherapy, another approach for improved local tumour control is the intrapleural application of different immunomodulating substances. One particularly promising approach is to stimulate innate immunity. Toll-like receptor (TLR) belong to the family of pattern recognition receptors (PRR) and ligation of these receptors by conserved motifs of microorganisms (pathogen-associated molecules) results in activation of the innate immune response. TLR9 ligands bind unmethylated CpG clusters. Both bacterial DNA and synthetic unmethylated CpG oligonucleotides have been shown to enhance cellular and humoral immunity against cancers via TLR-9. Furthermore experimentally CpG-ODNs were found to be potent enhancer of chemotherapy and radiotherapy and there-fore might also qualifly for multimodal treatment in mesothelioma.
In the underlying study we wanted to assess the effect of intratumoral injection of immunomodulatory agents + plus intrapleural chemotherapy loaded to a fibrin sealant (Vivostat®) on the volume and the incidence of tumour recurrence.
**Materials and Methods:** *Recurrence model:* A tumour cell suspension of 50 µl 1x$10^6$ rat malignant mesothelioma cells was inoculated subpleurally. Six days after inoculation, a tumour nodule of about 5mm in diameter was resected and animals were treated according to randomization after left-sided pneumonectomy and pleural abrasion: control (n=6), 500µg CpG-ODN (Cytosine-phosphate-guanosine-oligodeoxynucleotide) (n=6), Cisplatin-Vivostat® (n=6), Cisplatin-Vivostat®+500µg CpG-ODN (n=6). Primary endpoint was the volume of tumour recurrence 6 days after treatment. Secondary endpoints were the SRY-gene (sex-determining-region Y) expression for quantification of the ratio host/tumour cells into the local recurrence and cytokines express-ion profile in the tumour tissue by qPCR. Treatment-related toxicity was assess-ed by repeated blood samples.
**Results:** The volume of tumour recurrence was significantly reduced from 610mm3 in the control group to 11.7$mm^3$ in the Cisplatin-Vivostat® group (p=0.005) and to 21.8mm3 in the Cisplatin-Vivostat®+CpG group (p=0.003). The determination of SRY gene by qPCR-technique showed a higher ratio host/tumour cells in the Cisplatin-Vivostat®+CpG group (45/55%) compared to the Cisplatin-Vivostat® group (27/73%). Pro-inflammatory cytokines (IFN-gamma, IL-6, IL-12) were increased after treatment with Cisplatin-Vivostat®+CpG group. No significant treatment-related toxicity was observed.
**Conclusions:** Adjuvant treatment with chemo- and immunotherapy lead to significant reduction of mesothelioma recurrence after surgery in this aggressive tumour rat model. An additional effect of immunotherapy might be the recruitment of inflammatory cells at the site of tumour growth and concomitant cytokines secretion.
**Outlook:** Refinement of immunomodulatory intrapleural therapy and large-scale animal pharmacaodynamic experiments as preparation of human studies.

**Prognostic Marker for Malignant Pleural Mesothelioma**

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

**Background:** Malignant pleural mesothelioma (MPM) is a highly aggressive tumour with poor prognosis and limited response to therapy. MPM is characterized by complex chromosomal aberrations, including chromosome 10 losses. The tumour suppressor gene PTEN located on chromosome 10q23 plays an important role in different cancer, but it's relevance for MPM is unclear.

**Patients and Methods:** All malignant mesotheliomas, diagnosed between 1975 and 2004, were retrieved from the archives of the Zurich Pneumoconiosis Research Group, Switzerland. The total of 341 cases comprised 112 epithelioid, 183 biphasic and 46 sarcomatoid types. The tissue specimens were mainly derived from postmortem examination (77% autopsy, 23% biopsy) and had uniformly been formalin-fixed and paraffin-embedded. They had all been originally examined and classified for the histological subtype by one experienced lung pathologist and were reviewed to identify suitable areas for tissue microarray construction. The construction of a set of three tissue microarrays (TMA) was accomplished with a custom-made, semiautomatic tissue arrayer (Beecher Instruments, Sun Prairie, WI, USA) as described.

**Results:** Clinical data from 206 patients were available. 105 patients were stage T4 and 92 patients presented with regional and mediastinal lymph node metastasis. Loss of PTEN expression was observed in 62% of the cases. The survival time was correlated to PTEN expression in 126 cases with complete follow-up data. Comparing any PTEN expression versus no expression, median survival time was significantly longer (p= 0.0001) in patients with PTEN expression (15.5 months; 95% CI: 3.8; 27.2 versus 9.7 months; 95% CI: 7.9; 11.7). Cox-regression analysis revealed an association between PTEN expression and survival (p=0.003) independently from the histological subtype (p=0.7).

**Conclusion:** PTEN is an independent prognostic biomarker in mesothelioma patients. The frequent loss of expression of the tumour suppressor gene PTEN suggests involvement of the PI3K-AKT/protein kinase B (PKB) pathway in MPM, which may be relevant for future mesothelioma treatment.

**Outlook:** PTEN and other marker will be assessed in our prospective database of patients that underwent induction chemotherapy followed by extrapleural pneumonectomy.

## Achievements 2007

- Awards: Brompton Prize from the European Society of Thoracic Surgery, Leuven 06/07'
- Fellowships: Fellowship of European Society of Medical Oncology
- Grants: Zürcher Krebsliga

## Collaborations:

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

## Selected references:

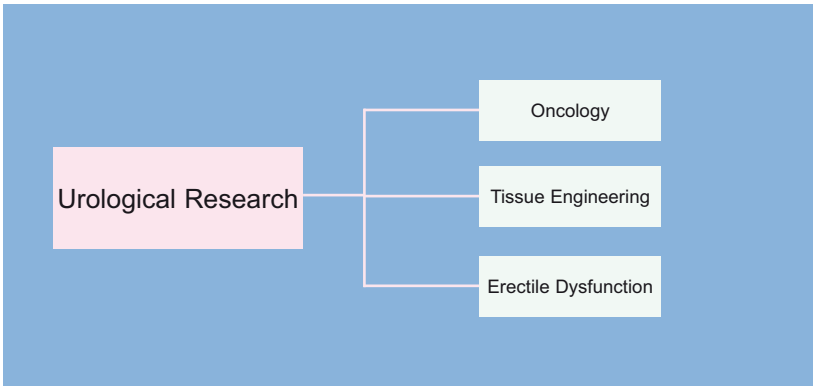

- Opitz I, Soltermann A, Abaecherli M, Hinterberger M, Probst-Hensch N, Stahel R, Moch H, Weder W. PTEN expression is a strong predictor of survival in mesothelioma patients. Eur J Cardiothorac Surg. 2008 Mar;33(3):502-6. Epub 2008 Jan 8.
- Opitz I, Sigrist B, Hillinger S, Lardinois D, Stahel R, Weder W, Hopkins-Donaldson S. Taurolidine and povidone-iodine induce different types of cell death in malignant pleural mesothelioma. Lung Cancer. 2007 Jun;56(3):327-36. Epub 2007 Mar 23.
- Opitz I, Lardinois D, Arni S, Hillinger S, Vogt P, Odermatt B, Rousson V, Weder W. Local recurrence model of malignant pleural mesothelioma for investigation of intrapleural treatment.
  Eur J Cardiothorac Surg. 2007 May;31(5):773-8. Epub 2007 Mar 12.
- Opitz I, Van der Veen H, Witte N, Braumann C, Mueller JM, Jacobi CA. Instillation of taurolidine/heparin after laparotomy reduces intraperitoneal tumour growth in a colon cancer rat model.
  Eur Surg Res. 2007;39(3):129-35. Epub 2007 Mar 2.
- Inderbitzin DT, Opitz I, Giger U, Kocher T, Krähenbühl L.
  Incidence of clinical pulmonary embolism after laparoscopic surgery.
  Br J Surg. 2007 May;94(5):599-603.
- Bobrich E, Braumann C, Opitz I, Menenakos C, Kristiansen G, Jacobi CA. Influence of intraperitoneal application of taurolidine/heparin on expression of adhesion molecules and colon cancer in rats undergoing laparoscopy.
  J Surg Res. 2007 Jan;137(1):75-82. Epub 2006 Nov 15.

## 2.6 Urological Research

Prof. Dr. med.
Tullio Sulser

Dr. med.
Maurizio Provenzano

Balmer Damina
Scientific Coordinator




### 2.6.1 Oncology

Dr. med.
Thomas Hermanns

MSc
Giovanni Sais

Dr. med.
Hans-Helge Seifert

Dr. med.
Maurizio Provenzano

**The prognostic value of correlations between lymph-angiogenesis, cancer metastasis and tumor staging in prostate cancer**
Dr. med T. Hermanns, MSc G. Sais, Dr. med. H.H. Seifert,
Dr. med. M. Provenzano

A number of angiogenesis factors have been indicated to play a role in the promotion of tumor spread in cancer patients. In particular, expression of vascular endothelial growth factors (VEGFs) and their receptors (VEGFRs) in urogenital cancers, particularly prostate cancer (PCa), has been suggested to determine a state of cancer invasion and dissemination. In many human cancers, the production of specific VEGFs by tumor cells and, eventually, by chemo-attracted macrophages, leading to an overwhelmed lymphatic vasculature within lymph-nodes, has been thus proposed as a target for a future tumor cell dissemination in early cancer stages. In PCa, VEGF-C and –D produced by both tumor cells and infiltrating macrophages, have been implicated in tumor lymph-angiogenesis and lymph-nodes metastasis through the activation of VEGFR-3 signaling pathways in lymphatic endothelial cells (LECs) and the expression of VEGF-C and VEGF-D has been strongly correlated to lymph-node metastasis in different retrospective studies. Recently, new investigations support also the hypothesis that lymph-angiogenesis could be initiated by direct targeting due to VEGF-C and/or -D and VEGFR-2 interactions. More recently, VEGF-A, a traditional blood vessel-specific growth factor, has been hypothesized to promote tumor lymph-angiogenesis and lymph-node metastasis through VEGFR-2 activation, thus representing the major signaling pathway in lymph-angiogenesis. However, the expression of these factors has not been comparatively evaluated in all different tumor grades and stages and their potential prognostic significance has not been fully explored. Furthermore, the roles of additional factors, such as insulin like growth factor 1 and 2 (IGF-1/2), platelet derived growth factor-BB (PDGF-BB), angiopoietin-1 (Ang1), fibroblast growth factor (FGF) and hepatocyte growth factor (HGF) in relation to VEGFs/VEGFRs remain still elusive.

**Collaborations:**

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

## Human polyomavirus BK and genitourinary tract malignancies

MSc G. Sais, Dr. med T. Hermanns, Dr. med. M. Provenzano

Human polyomavirus BK (BKV) is a DNA virus belonging to the Polyomaviridae family that also includes human polyomavirus JC (JCV), and Simian virus 40 (SV40). The virus is ubiquitous in the human population, establishing latent infections in the kidney and the genitourinary tract. Serological evidence indicates that nearly 90% of individuals are infected by early childhood, although a decrease in this rate during the human lifespan may be due to viral seroconversion (70-80%). Usually, polyomaviruses cause persistent sub clinical infections in humans and BKV infection rarely leads to clinical manifestations. However, when the immune system is compromised, as following transplantation, HIV infection or pharmacologic immunosuppression, rate and level of BKV replication increase and may lead to organ diseases (polyomavirus-associated nephropathy, PVAN). The polyomavirus genome consists of the non-coding control region, the early genes and the late genes. The polyomavirus-encoded early gene product large tumour antigen (LTag) has been identified early on as a key regulatory molecule. LTag interacts with a number of host cell molecules including the tumour-suppressor gene retino-blastoma family (Rb) products and p53. The BKV oncogenic effect appears to be highly associated to the latter LTag activity. Initially, the antigen binds to tumor suppressor proteins of the retinoblastoma family (pRb, p107, and p130) thereby interfering with their activity and inducing the infected cell to enter the cell cycle (phase S). Subsequently, and most importantly, the LTag inactivation of p53 prevents p53-mediated cell apoptosis of infected cell (Fig.1).

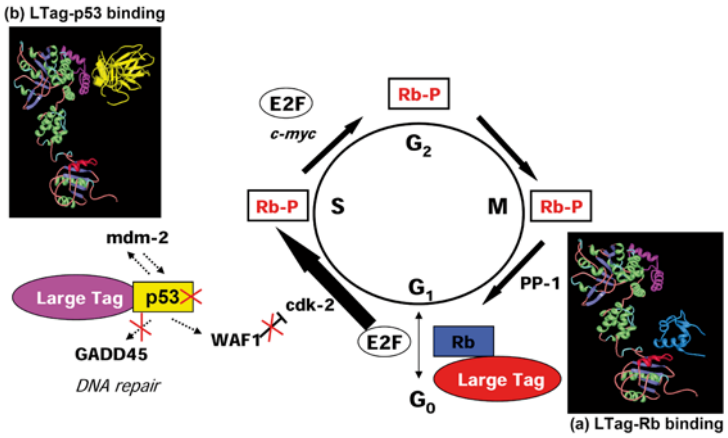



**Figure 1. The Large Tumor Antigen (LTag) interaction with cell cycle. (a)** Initially, the LTag binds **(red region)** to products of the Rb-family (pRb, p107, and p130 **in blue**) thereby interfering with their activity and inducing the infected cell to enter the cell cycle (phase S). **(b)** Subsequently, and most importantly, the LTag inactivation **(purple region)** of p53 **(in yellow)** allows the re-phosphorylation of pRb through the cyclin-dependent kinase (cdk) pathway and prevents the p53-mediated cell apoptosis of infected cell

This mechanism is used by the virus to keep the infected cells alive during the productive infection but in non-permissive cells it may lead to oncogenesis. Upon viral entry in non-permissive cells, in the context of an abortive infection, viral DNA is fully integrated in the host genome or resides in host cells as plasmid, thereby favouring the persistence of the virus without production of progeny virions. Thus, abortive infections may result in oncogenic transformation which has rendered polyomaviruses prototypes of DNA tumour virus well amenable to studies in experimental models, in particular the protein LTag. The role of human polyomaviruses in human cancer is still debated. Recent investigations have associated them with the outgrowth of specific cancer types including colorectal cancer, glioblastomas, mesotheliomas, and, possibly, lymphomas.

The involvement of BKV large Tag in the alteration of critical pathways of the human cell cycle together with the detection and expression of BKV large Tag sequences in preneoplastic prostate tissues prompt us to investigate the role of this viral antigen as target of cellular immune surveillance in genitourinary cancers, particularly prostate cancer. Our previous data suggest that cytotoxic T lymphocyte immune responses against LTag can be effectively expanded by reactivating T cell memory from BKV seropositive subjects using two newly identified HLA-A*0201 LTag peptides ($LTag_{406}$ and $LTag_{597}$) nesting within portions responsible for p53 binding ($LTag_{351–450}$ and $LTag_{533–626}$). The study also indicates that expanded HLA-A*0201 $LTag_{597}$ specific CTLs exquisitely belong to a CD45RA+/CCR7+(-) CD8+ T cell population. In addition 2–3 week cultures of separated cell populations indicated that specific CTL can only be generated from CD45RA+ cells. Importantly, even after these culture times, $LTag_{579}$ specific CD8+ T cells retain a CD45RA+/CCR7- phenotype, a relative infrequent event for CTL recognizing BKV unrelated peptides (Fig.2).

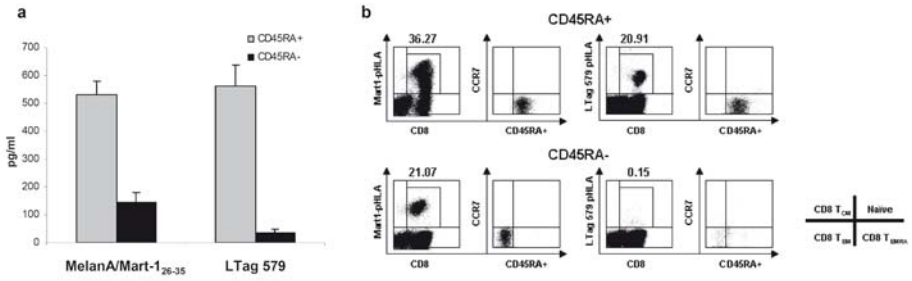



**Figure 2. BKV LTag 579 specific CD8+ T cells belong to the CD45RA+ subpopulation.** CD8+ T cells from HLA-A*0201+ BKV LTag seropositive donors were separated into CD45RA+ and CD45RA- fractions prior to stimulation with HLA-A*0201 restricted melanoma associated antigen MelanA/Mart-126–35 or BKV LTag 579 peptide. **(a)** After 3-week restimulations in the presence of cognate peptide-loaded autologous mature DCs, CD45RA+ and CD45RA- CD8+ T cell subpopulations IFN-γ protein release was tested by ELISA. **(b)** The CD8+ T cells phenotypes using MelanA/Mart-126–35 or BKV LTag 579 specific HLA-A*0201 multimers were also evaluated

## Achievements 2007

- Forschungskredit 2007, University of Zürich

## Collaborations:

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

## Selected references:


- Provenzano M, Bracci L, Wyler S, Hudolin T, Sais G, Gosert R, Zajac P, Palu' G, Heberer M, Hirsch HH, Spagnoli GC: Characterization of highly frequent epitope-specific CD45RA$^{+}$/CCR7$^{+/-}$ T lymphocyte responses against p53-binding domains of the human polyomavirus BK Large T antigen in HLA-A*0201+ BKV-seropositive donors. J Transl Med. 2006; 4(1): 47.
- Provenzano M, Panelli MC, Mocellin S, Bracci L, Sais G, Stroncek DF, Spagnoli GC, Marincola FM: MHC-peptide specificity and T cell epitome mapping: where immunotherapy starts. Trends Mol Med. 2006; 12(10): 465-472.
- Sais G, Wyler S, Hermanns T, Spagnoli GC, Sulser T, Provenzano M: Impairment of immune surveillance in polyoma BK virus seropositive prostate cancer (PCa) patients correlates to a T cell regulatory activity induced by Large T antigen (LTag)-p53 binding domains. (Abstract) 7$^{th}$ Day of Clinical Research Zürich.

## IDO expression and malignant transformation in prostate cancer

Dr. med. M. Provenzano, MSc G. Sais, Dr. med. T. Hermanns,
Dr. med. H.H. Seifert

A number of immunosuppressive factors have been suggested to play a role in functional impairment of the immune system in prostate cancer (PCa) patients. Among all, indoleamine 2,3-dioxygenase (IDO) has been considered to favour tumoral immune escape based on tryptophan degradation. We evaluated and compared the expression of genes encoding potential immunosuppressive factors such as IDO, ARG II and IL23 in human benign prostatic hyperplasia (BPH) and PCa also according to alpha-methylacyl-CoA racemace (AMACR) gene expression and kynurenine/tryptophan ratios. Furthermore, impairments of CD4+ and CD8+ T cells proliferation upon homeostatic cytokines (IL-2, IL-7, IL-15) stimulation was also quantified. 40 BPH and 36 PCa patients were enrolled upon informed consent. Quantitative RT-PCR was used to determine gene fold increases of immunosuppressive factors suggested to play a role for immune functional impairment in PCa. Peripheral blood mononuclear cells from same BPH and PCa patients or healthy donors were used for proliferative assays upon homeostatic cytokines stimulation. IDO protein expression was evaluated by immunohistochemistry. Tryptophan and its catabolites concentrations were evaluated in serum patients by HPLC. Statistic was performed using SPSS software package. IDO gene expression significantly correlated with both histological malignancy (p=0.004) and AMACR (p=0.001) accounting for almost 57% of PCa patients. Interestingly, $IDO^{high}$ gene expression (39% of IDO positive patients) was also correlating to serum kynurenine/tryptophan ratio (p=0.021) (Fig.1).

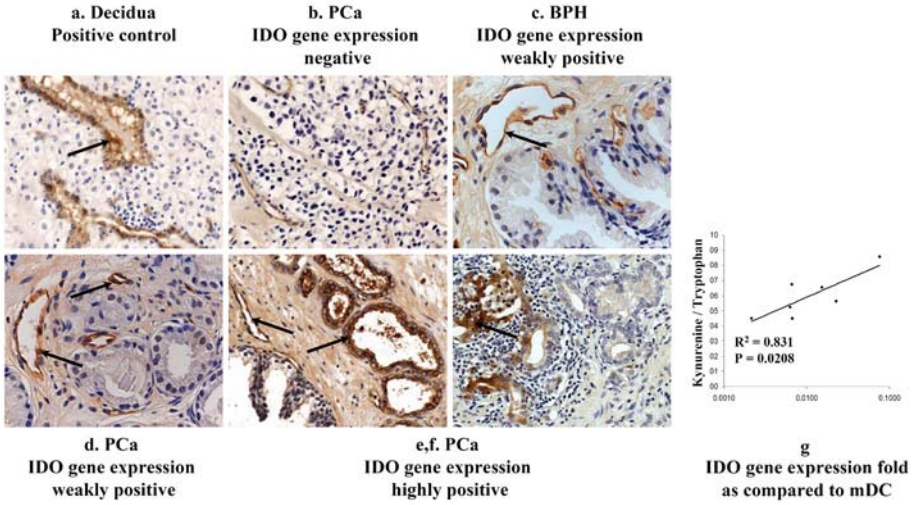



**Figure 1. Immuno-histochemical detection of IDO in PCA and BPH tissues and systemic impact of IDO-high gene expression in PCA tissues. (a.)** Decidua was used as positive control tissue and stained with IDO specific antibodies (400X). **(b.)** PCA tissue negative for IDO gene expression (400X). **(c.)** BPH tissue showing weak IDO gene expression, and **(d.)** PCA tissue showing weak IDO gene expression and displaying IDO+ endothelial cells in capillaries and IDO- tumor cells (630X). **(e,f)** PCA tissues expressing high levels of IDO gene expression and showing IDO+ tumour cells, especially in inflamed regions **(panel f)** (400X). Arrows indicate IDO+ cells. **(g)** A subgroup of PCA (n=9) tissues highly positive for IDO gene expression (IDOhigh) was identified. IDO gene expression in these tumours was correlated to kynurenine /tryptophan ratio in sera from the corresponding patients sampled simultaneously to collection of surgical specimens.

In contrast, ARG II, IL23, iNOS, eNOS genes were not significantly more expressed in PCa, as compared to BPH, while IL-17 expression was not modified. IL-6 gene expression was significantly (p=0.0002) enhanced while TGFb gene expression was significantly (p=0.035) decreased in PCa. Unfortunately, we could not find a significant reduction of T cells proliferation under homeostatic cytokine stimuli. IDO gene expression, frequently detectable in PCa and correlating with AMACR expression, appears to qualify as a marker of malignant transformation in prostate cancers. Further research is warranted to clarify its consequences on immune responsiveness in PCa patients.

**Collaborations:**

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

**Selected references:**

- Feder-Mengus C, Wyler S, Hudolin T, Ruszat R, Bubendorf L, Chiarugi A, Weber W, Bachmann A, Gasser T, Sulser T, Heberer M, Spagnoli GC, Provenzano M: Expression of IDO immunosuppressive factor gene and malignant transformation in prostate cancer. Eur Urol Suppl. 2008; 7(3):275 (Abstract 818).

## 2.6.2 Tissue Engineering for Urologic Tissues

Dr. med.,PhD
Daniel Eberli

Dr. med.
Lukas Hefermehl

**Bioengineered Muscle for Functional Sphincter Reconstruction**
Dr. med. Daniel Eberli, Dr. med. Lukas Hefermehl

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

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

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

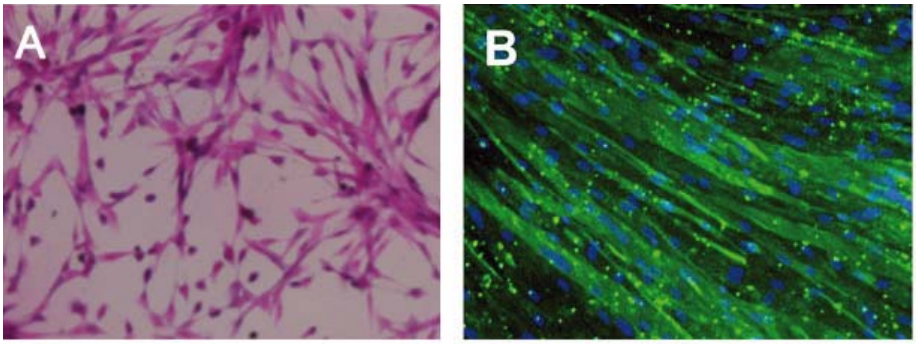



Figure 1. A) Human muscle precursor cells 7 days after plating. Giemsa Stain.
B) Immunohistochemistry using anti-desmin at passage 3. After eight days in fiber formation medium the muscle precursor cells fused, formed myofibers and aligned. Magnification 200x.

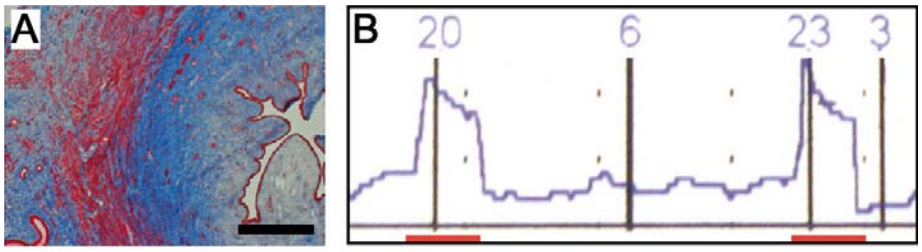



Figure 2. A) Maison's trichrome staining showing the morphological advances of MPC injected animals with reconstruction of the muscle layer in the sphincter area. Stain showes muscle cells and mucus in red and extracellular matrix in blue.
B) Functional assessment of the engineered muscle tissue in vivo. The figure shows the sphincter pressure rise upon stimulation of the pudendal nerve.

## Collaborations:

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

## 2.6.3 Erectile Dysfunction

Dr. med.
Alexander Müller

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

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

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

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

This model seems to be representative of neural injury that occurs at the time of pelvic surgery and thus, allow the assessment of the neuromodulatory properties of pharmacologic strategies in a pre-clinical fashion prior to human clinical trials.

**D. Preliminary Studies**

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

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

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

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

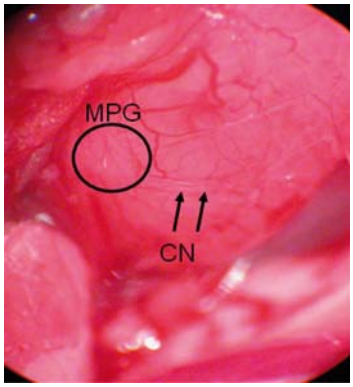



Figure 1: Cavernous Nerve in the Rat
Intra-operative picture showing the cavernous nerve (CN) coming from the major pelvic ganglion (MPG) and running along the surface of the prostate in the rat.

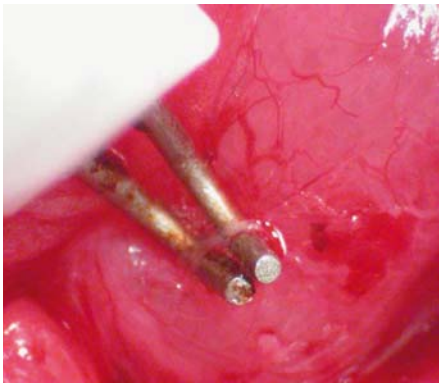


Figure 2:
Intra-operative picture displaying the cavernous nerve (CN) hooked up by an electrode for electrical stimulation to measure the maximum intracavernosal pressure (ICP). At the same time of CN simulation the corresponding mean arterial blood pressure (MAP) will be reported as the ICP/MAP ratio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

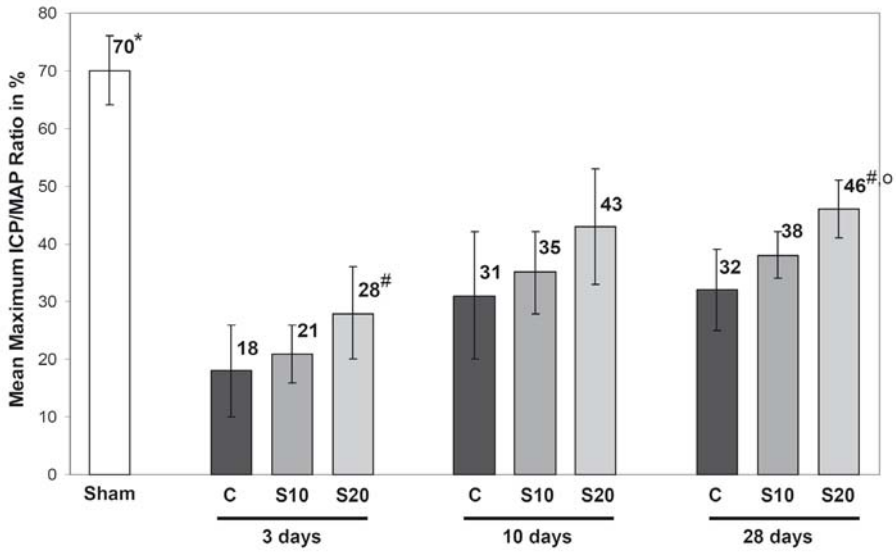



Figure 3: Functional Results
Graph showing the mean maximum intracavernosal pressure (ICP) divided by the corresponding mean arterial pressure (MAP), reported as ICP/MAP ratio as a percentage for Control (bilateral CN crush), and both treatment groups S10 (daily 10 mg/kg sildenafil sc) and S20 (daily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

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

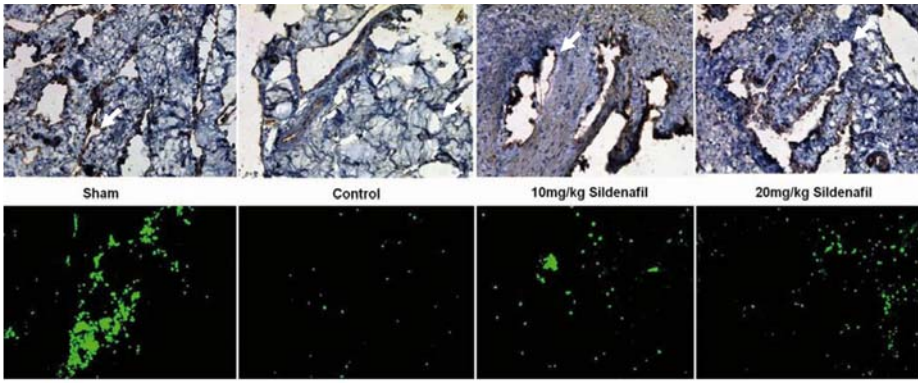



Figure 4: Immunohistochemistry staining for CD31
At 28 days after cavernous nerve injury the Control group (bilateral CN crush) demonstrated a lower density of CD31 staining compared to Sham (no CN crush) and both treatment groups S10 und S20 displayed higher staining compared to control on both immunohistochemistry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

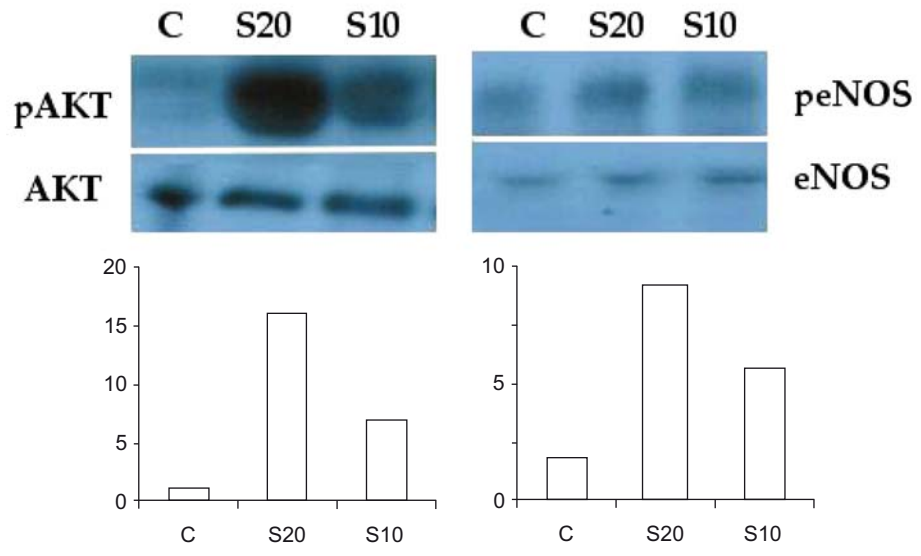



Figure 5: Immunoblotting for eNOS/AKT
Both treatment groups S10 and S20 (10 and 20 mg/kg sildenafil sc daily) demonstrated greater activation (phosphorylation) of AKT and eNOS compared to the Control group C.

## Achievements 2007

- Muller A, Donohue J, Mullerad M, Tal R, Kobylarz P, Li P, Scardino P, Sulser T, Mulhall JP. The functional and structural consequences of cavernous nerve injury are ameliorated by sildenafil citrate. Presented at the 63rd Swiss Congress of Urology, 27.-29. September 2007, Basel, Switzerland
- Muller A, Donohue J, Tal R, Mullerad M, Kobylarz K, Paduch D, Scardino P, Sulser T, Mulhall JP. Sildenafil and cavernous nerve injury in the rat: defining the optimal dosing and timing regimen. Presented at the 63rd Swiss Congress of Urology, 27.-29. September 2007, Basel, Switzerland
- Muller A, Parker M, Waters BW, Flanigan RC, Mulhall J. Analysis of predictors of outcomes with pharmacological penile rehabilitation following radical prostatectomy. Presented at the 63rd Swiss Congress of Urology, 27.-29. September, Basel 2007, Switzerland
- Muller A, Tal R, Donohue J, Akin-Olugbade Y, Kobylarz K, Sulser T, Mulhall J. The effect of hyperbaric oxygen therapy on erectile function recovery in the rat cavernous nerve injury model. Presented at the 63rd Swiss Congress of Urology, 27.-29. September 2007, Basel, Switzerland
- Muller A, Kobylarz K, Humphreys S, Weyman C, Mulhall JP. Microarray analysis of the impact of sildenafil citrate treatment on gene expression in the rat cavernous nerve injury model. Presented at the Annual Meeting of the American Society of Urology, 19.-24. May 2007, Anaheim, Californian, USA
- Muller A, Donohue JF, Golijani D, Tal R, Aking-Olugbade Y, Kobylarz K, Cohen-Could L, Bennett N, Scardino PT, Mulhall JP. FK506 and erectile function preservation in the cavernous nerve injury model: optimal dosing and timing. Presented at the 10th Meeting of the European Society of Sexual Medicine, 25.-28. November 2007, Lisbon, Portugal
- Muller A, Tal R, Donohue J, Akin-Olugbade Y, Kobylarz K, Scardino P, Mulhall JP. The effect of hyperbaric oxygen therapy on erectile function recovery in the rat cavernous nerve injury model. Presented at the 10th Meeting of the European Society of Sexual Medicine, 25.-28. November 2007, Lisbon, Portugal
- Muller A, Rojas Cruz C, Choi JM, Nelson C, Mulhall JP. Predictors of venous leak development in men following radical prostatectomy. Presented at the Meeting of the Sexual Medicine Society of North America, 5.-9. December 2007, Chicago, USA
- Muller A, Guhirng P, Mulhall JP. Can intracavernosal injection therapy salvage phosphodiesterase type 5 inhibitor failures? Presented at the Meeting of the Sexual Medicine Society of North America, 5.-9. December 2007, Chicago, USA

**Collaborations:**

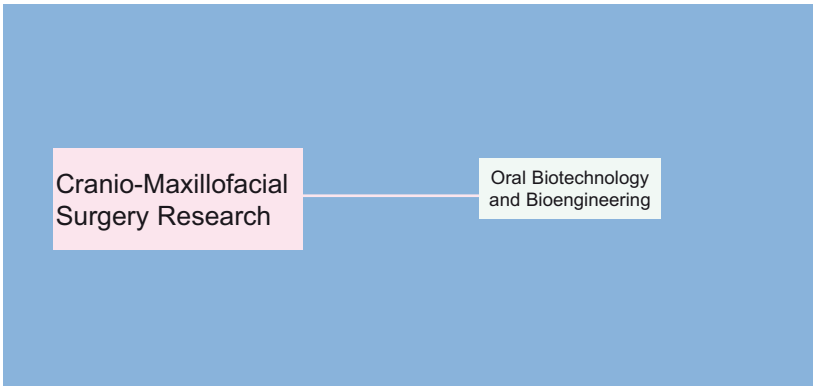
- Prof. J.P. Mulhall, Laboratory of Sexual Medicine, Weill Medical College of Cornell University and Memorial Sloan-Kettering Cancer Center, New York, NY, USA.
- PD Dr. R. Graf, Division of Visceral & Transplant Surgery, USZ, Zürich

**Selected references:**


- Muller A*, Mulhall JP*, Donohue JF, Mullerad M, Tal R, Kobylarz KA, Paduch DA, Li P, Scardino PT. (*Equally contributed). The functional and structural consequences of cavernous nerve injury are ameliorated by sildenafil citrate. Accepted for publication at Journal of Sexual Medicine
- Muller A*, Tal R*, Donohue JF, Akin-Olugbade Y, Kobylarz K, Paduch D, Cutter S, Mehrara B, Scardino PT, Mulhall JP. The effect of hyperbaric oxygen therapy (HBOT) on erectile function recovery in the rat cavernous nerve injury model. In press at Journal of Sexual Medicine
- Muller A*, Mulhall JP*, Donohue JF, Golijani D, Tal R, Akin-Olugbade Y, Kobylarz KA, Cohen-Gould L, Bennett NE, Scardino PT. (*Equally contributed). FK506 and erectile function preservation in the cavernous nerve injury model: optimal dosing and timing. Accepted for publication at Journal of Sexual Medicine
- Muller A, Akin-Olugbade Y, Deveci S, Donohue JF, Tal R, Kobylarz KA, Palese M, Mulhall JP. The impact of shock wave therapy at varied energy and dose levels on functional and structural changes in erectile tissue. In press at Journal of European Urology
- Müller A, Müntener M. Erektile Dysfunktion - Aktuelle Stufentherapie und Koexistenz mit Herz-Gefäss-Erkrankungen und metabolischem Syndrom. The Medical Journal 2007 Nov; 7: 22-27.
- Muller A, Smith L, Parker M, Mulhall JP. Analysis of the efficacy and safety of sildenafil citrate in the geriatric population. BJU Int. 2007 Jul;100(1):117-21.
- Muller A, Shelton J, Parker M, Guhring P, Mulhall JP. Nitrate cessation profiles in men wishing to use sildenafil citrate. Urology. 2007 May; 69(5):946-9.
- Muller A, Mulhall JP. Erection, Emission, and Ejaculation: Mechanisms of Control. Infertility in the Male, 4th Edition by Lipschutz, Howards and Niederberger. Cambridge University Press, Publication 2007

## 2.7 Cranio-Maxillofacial Surgery Research

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




### 2.7.1 Oral Biotechnology and Bioengineering

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

Martin Ehrbar
PhD

Prof. PhD
Heinrich Walt

Dr. med
Astrid Kruse

Ana Sala
PhD-student

Rita Correro
PhD-student

Patrick Hänseler
PhD-student

Yvonne Bloemhard

Alexander Tchouboukov

**BMP and bone regeneration**
Franz Weber, Martin Ehrbar, Chafik Ghayor, Blanca San Miguel, Ana Sala, Rita Correro, Patrick Hänseler, Alexander Tchouboukov, Yvonne Bloemhard, Astrid Kruse, Heinrich Walt

In the early 60ies of the last century, Marshall Urist demonstrated the existence of a bone inducing principle. From there it took more than 26 years until the proteins responsible for osteoinduction: the bone morphogenetic proteins (BMPs) were isolated to purity, cloned and expressed recombinantly. Another 13 years had to pass till the FDA approved the use of recombinant human BMP-2 for spinal fusion. Unfortunately the amount needed for clinical applications is very high. 6 mg rhBMP-2, 100 times the natural BMP content in humans is applied to induce spinal fusion between two levels. The goal of our research is to develop novel delivery systems aiming towards a reduction of the amount of BMP needed. Another strategy, recently developed in our laboratory is the use of agents which multiply the biological effect of BMP. For the future we hope to combine the use of novel delivery systems and synergistic agents, which in the end could reduce the risk and the costs for the clinical application of BMPs.

**Synthetic hydrogels**
Biomaterial scaffolds that support and guide cell infiltration are a prerequisite for tissue regeneration and repair. Scaffolds formed of naturally occurring polymers such as collagen or fibrins have been used successfully for the induction of angiogenesis and bone regeneration. However their biological and physical properties are mainly dependant on composition and concentration of the components and cannot be varied individually. Engineered artificial Extracellular Matrix (aECM) that can readily be adapted to physical demands and regulate specific cell behavior would enable the regeneration or de novo formation of tissues inside the body or from tissue culture. Despite reported progress on such materials is impressive, the bioactivity of state of the art aECM is too reductionistic compared to collagen or fibrin based gels.

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

**Bone substitute materials**

*1) Three Dimensionally Designed Cell Cultures Consisting of Micro-structured Cell-sheets and Polymer Layers for Tissue Engineering:*

Tissue engineering is emerging as a significant potential alternative or complementary strategy whereby tissue and organ failure is addressed by implanting natural, synthetic, or semi-synthetic tissue and organ mimics that are functional from the start or that grow into the required functionality. Recent progresses in cell-sheet engineering suggest a promising clinical potential of reconstructed tissues for transplantation. Novel cell culture surfaces based on temperature-responsive polymers allow for the non-invasive harvesting of viable contiguous cell sheets that can be readily used in surgical procedures. This technique has been successfully applied to various tissue reconstructions, including ocular surfaces, periodontal ligaments, cardiac patches and bladder. However, the use of cell sheets so far has been limited to thin structures (up to five cell layers) because of the problems with non-sufficient oxygen and nutrition supply to the cells localized inside of the engineered tissue.

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

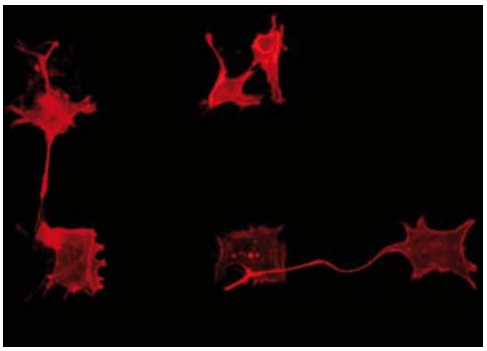
*2) Improvements of Synthetic bone blocks for vertical bone augmentation*

The goal of this subproject is the development of suitable in vitro models for 3D-cell culture and angiogenesis in synthetic bone blocks for vertical bone augmentation and huge bony defects in the cranio-maxillofacial area and to compare the effect of various modifications on cell behaviour (penetration-survival-differentiation-activation) and on bone regeneration in an in vitro animal model.

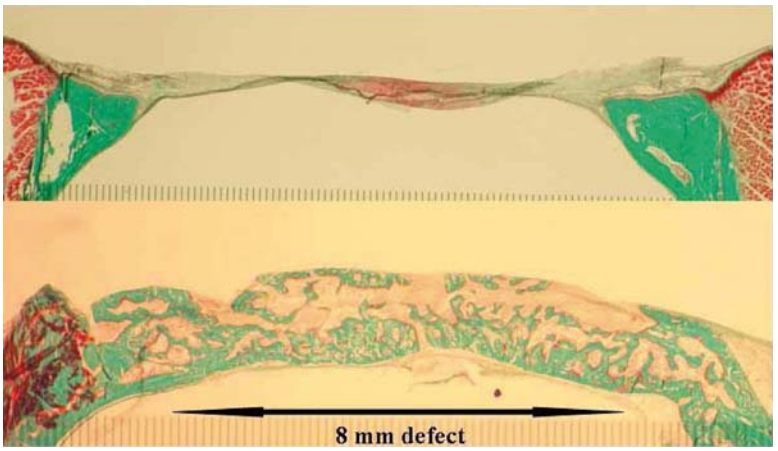
*3) Porous bioactive glass as bone substitute material*

“bioactive” substances, capable of forming a bone-like apatite layer on their surface, similar to our naturally occurring bone hydroxyapatite. Some of the main bioactive ceramics used clinically are: bioactive glass within the Na2O-CaO-SiO2-P2O5 system, hydroxyapatite and sintered -tricalcium phosphate. Out of this group, bioactive glass has been furthermore shown to promote osteo-blastic differentiation in vitro, as well as osteoinduction in vivo; therefore,there is a great interest to study its potential in bone regeneration.

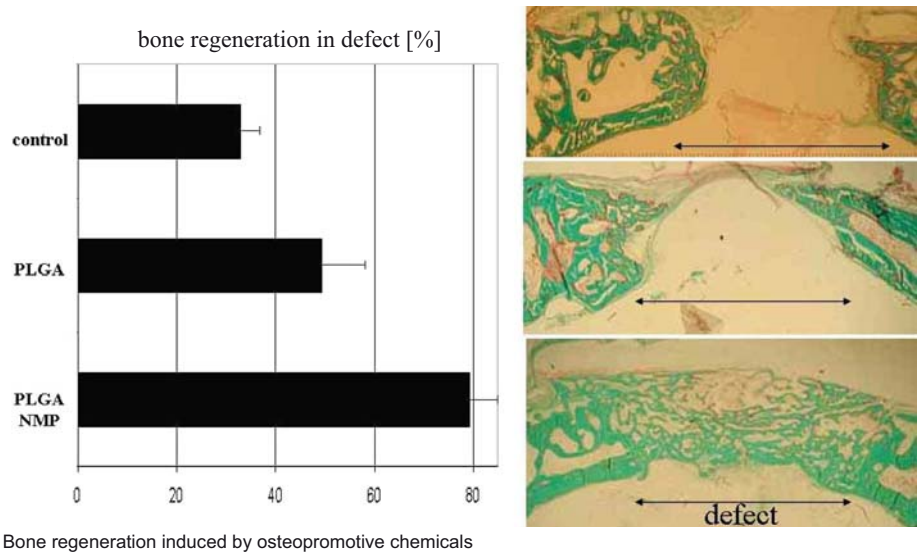
there is a great interest to study its potential in bone regeneration.
Our study is aimed at a glass with the following composition: 53% SiO2, 20% CaO , 6% Na2O, 12% K2O, 5% MgO, and 4% P2O5 (w/v), produced in the shape of fibers, which are further sintered into a final scaffold with 70% porosity. Since the porous structure of this sintered glass is unique we want to evaluate its potential in bone tissue engineering.



Cell guidance by surface modifications




Bone regeneration by the use of bone morphogenetic protein




Bone regeneration induced by osteopromotive chemicals

**Mechanobiology of cartilage and cartilage tissue engineering**

Our objective is to gain knowledge of the mechanobiology of temporomandibular joint (TMJ) cartilage, by designing functional tests of TMJ disc tissue and cartilage tissue in general. The long-term objective of this research is to understand the pathomecha-nics of TMJ degeneration. This interdisciplinary research can be extended to other joints, such as e.g. the knee, also yielding functional tests for synthetic or tissue engineered replacement materials. The functional tests will be performed by means of a self-developed cartilage explants mechanical testing system that will reproduce physiological and pathological conditions on live tissue. A second aspect of this research is cartilage tissue engineering by using this mechanical testing system as mechanical stimulator of cartilage tissue engineered in our synthetic matrices.

**Nanomedicine**

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

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

## Achievements 2007

- Jung R.E. , Hammerle C.H.F., Kokovic V., Weber F.E. (2007) Bone regeneration using a synthetic matrix containing a PTH peptide combined with a grafting material
  Int J Oral Maxillofac Impl 22(2) 258-266.
- Ehrbar M., Rizzi S.C., Hlushchuk R., Djonov A., Zisch A. Hubbell J.A., Weber F.E., Lutolf M. (2007) Enzymatic formation of modular cell-instructive fibrin analogs for tissue engineering
  Biomaterials 28(26): 3856-3866.
- Ehrbar M, Rizzi S., Schoenmakers R, SanMiguel, B., Hubbell JA; Weber FE Lutolf, M (2007) Biomolecular hydrogels formed and degraded via site-specific enzymatic reactions
  Biomacromolecules 8(10): 3000-3007
- Jung R.E., Weber F.E. , Thoma D.S., Ehrbar M., Cochran D.L., Hammerle C.H.F. (2007) "BMP-2 enhances bone formation when delivered by a synthetic matrix containing HA/TCP"
  Clin Oral Impl Res 19:188-195
- Wechsler S., Fehr D., Molenberg A., Raeber G., Schense J.C., Weber F.E. (2007) A novel, tissue occlusive poly(ethylene glycol) hydrogel material
  J Biomed. Mat. Res. A Aug 9; [Epub ahead of print]

## Collaborations:

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

**Selected references:**


- Lutolf, M.P., Weber,F.E., Schmoekel, H.G., Schense, J.C., Kohler, T., Müller, R. and J.A. Hubbell (2003) Repair of bone defects using synthetic mimetics of collagenous extracellular matrices. Nature Biotechnol. 21: 513-518
- Lutolf, M.P., Lauer-Fields J.L., Schmoekel H.G., Metters A.T., Weber F.E., Fields G.B., Hubbell J.A. (2003) Synthetic MMP-sensitive hydrogels for the conduction of tissue regeneration: Engineering cell-invasion characteristics Proc. Natl. Acad. Sci (USA) 100: 5413-5418
- Jung R. E., Glauser R., Schaerer P., Hammerle C.H.F., Sailer H.F., Weber F. E. (2003) The effect of rhBMP-2 on guided bone regeneration in humans. A randomized, controlled clinical and histomorphometric study. Clin. Oral Impl. Res. 14:556-568
- Weber F.E., Schmoekel H.G., Oelgeschläger M., Nickel J., Maly F., Hortschansky P., Grätz, K.W. (2003) Deletion mutants of BMP folding variants act as BMP antagonists and are efficient inhibitors for heterotopic ossification J. Bone Miner. Res. 18:2142-2151
- Keller O.R., Weber F.E., Grätz K.W., Baltensperger M.M., Eyrich G.K. (2003) Laser induced temperature changes in dentin J. Clin. Laser Med. Surg. 21:375-381
- Schmoekel H., Schense J.C., Weber F.E., Grätz K.W., Gnägi D., Müller R., Hubbell J.A. (2004) Bone healing in the rat and dog with nonglycosylated BMP-2 demonstrating low solubility in fibrin matices. J Orthopaed. Res. 22:376-381

## 2.8. Surgical Intensive Care Medicine

PD Dr. med.
John F. Stover

Prof. Dr.
Reto Stocker

Silke Ludwig

Jutta Sommerfeld

Angela Fendel

**Integration of multimodal monitoring in daily clinical routine improves treatment of patients with severe traumatic brain injury**

**Additional damage following severe traumatic brain injury**
Severe traumatic brain injury (TBI) is associated with a plethora of different secondary cascades which can induce and aggravate underlying brain injuries. In this context, it is of high importance to avoid hypoxia, hypotension, fever, and hyperglycemia.
Existing brain damage increases intracranial pressure (ICP) which, in turn, can induce further injuries related to impaired cerebral perfusion. To date, official guidelines suggest to commence specific treatment options whenever ICP reaches pathologic values (approx. 20 mmHg). This, however, supports the misconception that pathologic alterations are absent whenever the ICP is normal (< 15 mmHg). Our own experimental as well as clinical data clearly show that a more aggressive and faster approach is indispensable to correct metabolic deterioration following severe TBI since these alterations do occur at normal ICP values.

**Need to commence aggressive monitoring and treatment early after TBI**
Based on our own experimental data we could show that pathologic changes promoting cell death occur at normal ICP levels and even in absence of known secondary insults as e.g., hypoxia, hypotension, fever, and metabolic disturbances. Consequent multimodal monitoring started immediately upon arrival on the intensive care unit (ICU) even at normal ICP values (< 15 mmHg) repeatedly unmasked patients with signs of cerebral metabolic perturbation. These alterations could then be corrected easily by simply increasing the depth of sedation using thiopental (fig. 1) and adjusting the ventilator settings.

**Unmasking otherwise occult alterations in cerebral activity, metabolism and perfusion**
Measuring ICP alone has become insufficient in the treatment of patients suffering from severe TBI. The ICP is a good indicator for large edema formation and space occupying lesions but is insufficient in unmasking metabolic changes. If these changes remain occult, deteriorations are allowed to occur resulting in elevated ICP which then can become difficult to treat.
**EEG activity:** Application of specialized electrodes to the patients' forehead and continuous measurement of EEG activity allows to determine the actual depth of sedation and is used to adjust dosage of analgetics and sedatives with the aim of optimizing pharmacological coma to avoid insufficient as well as excessive drug infusion.
**Jugular venous catheters:** Insertion of a catheter in the jugular vein is used to assess global changes in perfusion, oxygenation, and metabolism within the brain. Changes in oxygen saturation as well as differences in lactate, glucose, and bicarbonate determined in paired arterial and jugular venous samples unmask intracerebral deterioration and allow to guide therapeutic interventions (fig. 2).

**ptiO2:** Insertion of specialized catheters into the frontal lobe is used to determine changes in brain tissue oxygen. Changes in ptiO2 reflect alterations in cerebral perfusion as well as oxygen delivery and oxygen consumption allowing to adjust therapeutic interventions as e.g., cerebral perfusion pressure (fig. 2), FiO2, ventilation, transfusion, etc. Sufficient ptiO2 levels significantly reduced the number and frequency of transfusions.

**Microdialysis:** Insertion of specialized catheters into the frontal lobe is used to measure changes in extracellular glucose, lactate, pyruvate, glutamate, and glycerol which are then analyzed enzymatically at bed-side. Extracellular fluid is dialyzed in 60 minute intervals until removal of these catheters. These measurements complement other neuromonitoring techniques and guide further diagnostic measures as e.g., EEG analysis and transcranial Doppler to assess seizure activity and/ or vasospasm (fig. 3).

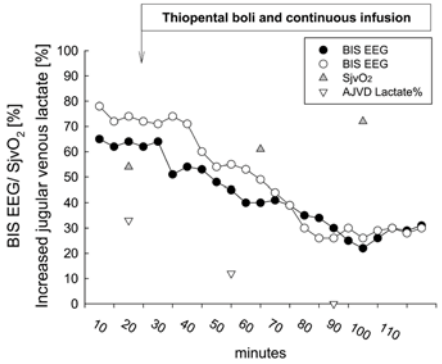



Figure 1
Increased EEG activity (BIS EEG > 50) coincided with cerebral metabolic impairment reflected by elevated $SjvO_2$ and pathologic arteriojugular venous difference in lactate levels despite normal ICP values (< 15 mmHg). Administration of thiopental corrected these signs of metabolic perturbation.

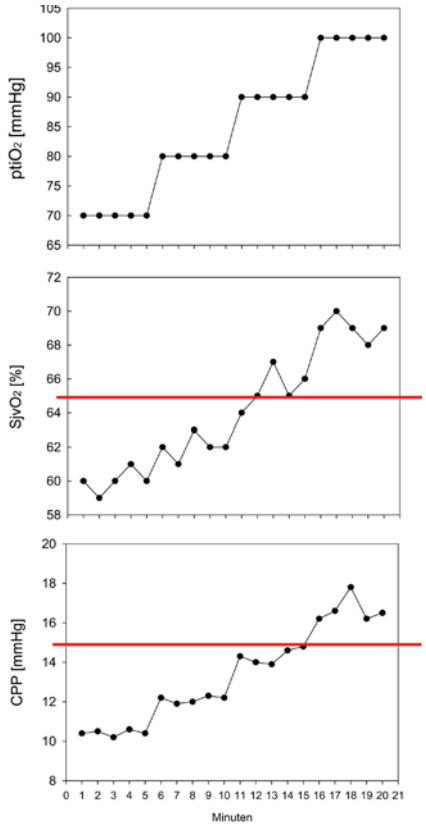



Figure 2
$SjvO_2$ and $ptiO_2$ values unmask insufficient cerebral perfusion. Controlled increase in mean arterial blood pressure significantly increased cerebral perfusion pressure (CPP) and improved cerebral oxygenation.

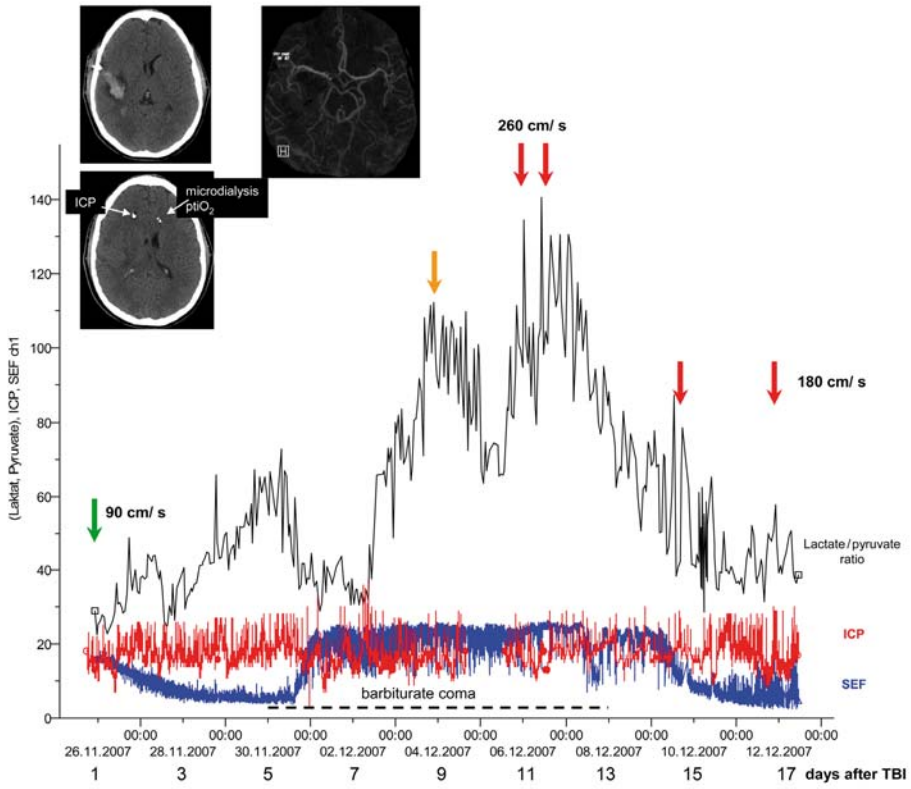



Figure 3
Changes in lactate/ pyruvate ratio preceeded occurrence and reversal of cerebral vasospasm following severe traumatic brain injury.

## Achievements 2007

- Grant approval by the Hartmann Müller Stiftung

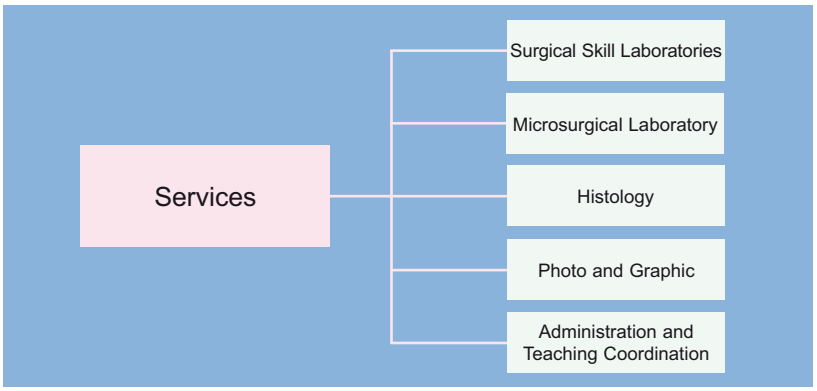
## Collaborations:

- Dr. med. Lars Asmis, Institut für Klinische Hämatologie
- PD Dr. Marius Keel, Dr. rer. nat. Luc Härter, Ursula Steckholzer, Klinik für Unfallchirurgie
- PD Dr. Klaus Schaser, Klinik für Unfallchirurgie, Charité Berlin
- Prof. Dr. Unterberg, Klinik für Neurochirurgie, Heidelberg

## Selected references:


- Thomale UW, Bender M, Casalis P, Rupprecht S, Griebenow M, Neumann K, Woiciechowsky C, Unterberg AW, Stover JF. Tacrolimus depresses local immune cell infiltration but fails to reduce cortical contusion volume in brain-injured rats. Immunobiology. 2007;212(7):567-76.
- Thomale UW, Griebenow M, Mautes A, Beyer TF, Dohse NK, Stroop R, Sakowitz OW, Unterberg AW, Stover JF. Heterogeneous regional and temporal energetic impairment following controlled cortical impact injury in rats. Neurol Res. 2007 Sep;29(6):594-603.
- Stover JF, Steiger P, Stocker R. Neostigmine-induced increase in EEG activity following severe traumatic brain injury. J Neurosurg Anesthesiol. 2007 Apr;19(2):143-4.
- Sakowitz OW, Stover JF, Sarrafzadeh AS, Unterberg AW, Kiening KL. Effects of mannitol bolus administration on intracranial pressure, cerebral extracellular metabolites, and tissue oxygenation in severely head-injured patients. J Trauma. 2007 Feb;62(2):292-8.
- Schaser KD, Disch AC, Stover JF, Lauffer A, Bail HJ, Mittlmeier T. Prolonged superficial local cryotherapy attenuates microcirculatory impairment, regional inflammation, and muscle necrosis after closed soft tissue injury in rats. Am J Sports Med. 2007 Jan;35(1):93-102.

# 3. Services





Boris Leskosek


Alush Avdyli

## 3.1 Surgical Skill Laboratories

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


Vlasta Strohmeier

## 3.2 Microsurgical Laboratory

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


Astrid Morger

## 3.3 Histology

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

## 3.4 Photo and Graphic Services

Nico Wick,
Photographer

Lea Schütz-Cohen,
Photographer

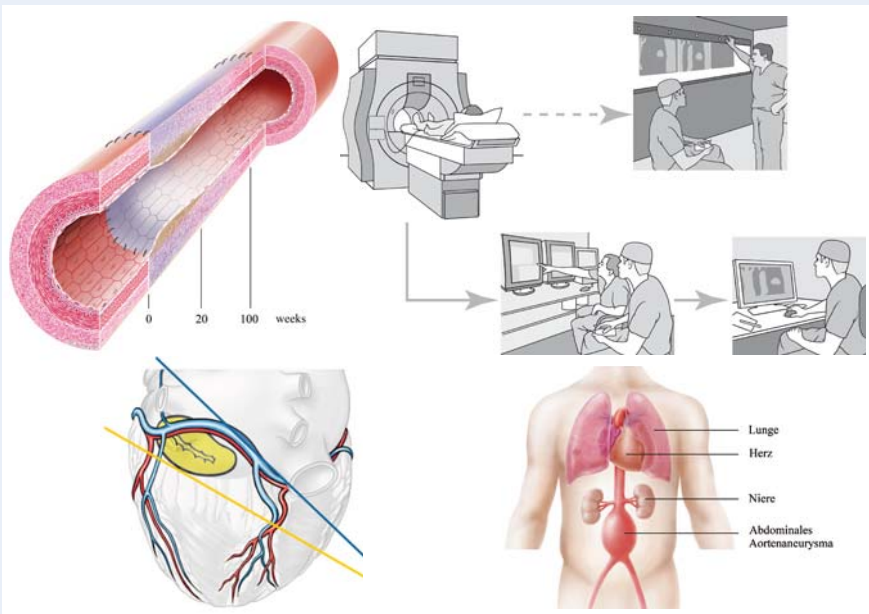
Stefan Schwyter,
Scientific
Illustrator

Carol De Simio,
Scientific
Illustrator

A quick, flexible, versatile and professional service.

We offer

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



Juliana Brink-Bogo
Administration
Division of Surgical Research

Susanne Frehner
Administration
Division of Surgical Research

## 3. 5 Administration

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

Corinne Renold,
Teaching Coordination
Division of Surgical Research

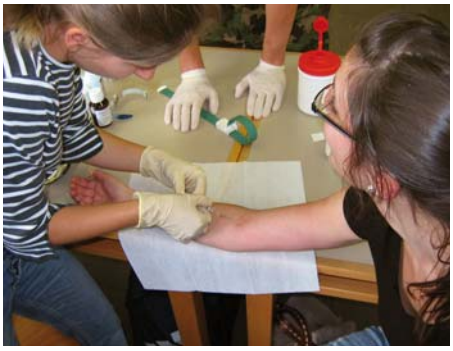
## 3. 6 Teaching Coordination

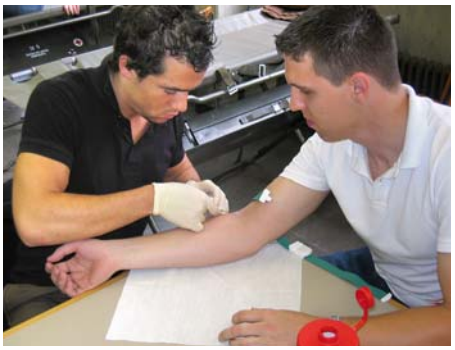
- Coordination and organization of the learning and teaching units in the Department of Surgery from 1$^{st}$ to 6$^{th}$ years of study including lectures and clinical courses in the compulsory part of the curriculum as well as in the electives; excluded are the clinical rotations during the 5$^{th}$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

# 4. Events and Workshops at the Division of Surgical Research in 2007



Microsurgical laboratory course





Injection class for medical students


Christmas party

# 5. Publications 2007


- Clavien PA, Petrowsky H, DeOliveira ML, et al. Strategies for safer liver surgery and partial liver transplantation. N Engl J Med 2007; 356:1545-1559
- Contaldo C, Meier C, Elsherbiny A et al: Human recombinant erythropoietin protects the striated muscle microcirculation of the dorsal skinfold from post-ischemic injury in mice. Am J Physiol Heart Circ Physiol. 2007; 293: H274-83
- Duenschede F, Erbes K, Kircher A, Westermann S, Schad A, Riegler N, Ewald P, Dutkowski P, Kiemer AK, Kempski O, Junginger T. Protection from hepatic ischemia/reperfusion injury and improvement of liver regeneration by alpha-lipoic acid. Shock 2007;27:644-51
- Duenschede F, Westermann S, Miesner I, Albrecht-Schock S, Kneist W, Korenkov M, Schad A, Dutkowski P, Kiemer AK, Junginger T. Hepatocellular injury of nonischemic liver tissue after selective clamping in rats--protective action by pharmacological pretreatment with lipoic acid. Eur Surg Res 2007;39:325-31
- Ehrbar M, Rizzi S.C, Hlushchuk R, Djonov A, Zisch A, Hubbell J.A, Weber F.E, Lutolf M. Enzymatic formation of modular cell-instructive fibrin analogs for tissue engineering. Biomaterials 2007; 28(26): 3856-3866
- Ehrbar M, Rizzi S, Schoenmakers R, SanMiguel B, Hubbell JA, Weber FE, Lutolf M. Biomolecular hydrogels formed and degraded via site-specific enzymatic reactions. Biomacromolecules 2007; 8(10): 3000-3007
- El-Badry AM, Graf R, Clavien PA. Omega 3 - Omega 6: What is right for the liver? J Hepatol 2007; 47:718-725
- El-Badry AM, Moritz W, Contaldo C, et al. Prevention of reperfusion injury and microcirculatory failure in macrosteatotic mouse liver by omega-3 fatty acids. Hepatology 2007; 45:855-863
- Georgiev P, Clavien PA, Gores GJ. Liver graft protection by antiapoptotic drugs: a step further. Liver Transpl 2007; 13:318-320
- Georgiev P, Navarini AA, Eloranta JJ, et al. Cholestasis protects the liver from ischaemic injury and post-ischaemic inflammation in the mouse. Gut 2007; 56:121-128
- Grosjean MB, Lenzlinger PM, Stahel PF, Yatsiv I, Shohami E, Trentz O, Kossmann T, Morganti-Kossmann MC. Immunohistochemical characterization of Fas (CD95) and Fas Ligand (FasL/CD95L) expression in the injured brain: relationship with neuronal cell death and inflammatory mediators. Histol Histopathol 2007;22:235-50.
- Handschin AE, Trentz OA, Hemmi S, Wedler V, Trentz O, Giovanoli P, Wanner GA. Leptin increases extracellular matrix mineralization of human osteoblasts from heterotopic ossification and normal bone. Ann Plast Surg 2007;59:329-33.
- Inci I, Zhai W, Arni S, et al. N-acetylcysteine attenuates lung ischemia-reperfusion injury after lung transplantation. Ann Thorac Surg 2007; 84:240-246; discussion 246
- Inci I, Zhai W, Arni S, et al. Fibrinolytic treatment improves the quality of lungs retrieved from non-heart-beating donors. J Heart Lung Transplant 2007; 26:1054-1060
- Jung R.E, Hammerle C.H.F, Kokovic V, Weber F.E. Bone regeneration using a synthetic matrix containing a PTH peptide combined with a grafting material.

Int J Oral Maxillofac Impl 2007; 22(2) 258-266.
- Keel M, Meier C. Chest injuries - what is new? Curr Opin Crit Care 2007; 13:674-9
- Kelm JM, Moritz W, Schmidt D, Hoerstrup SP, Fussenegger M. In vitro vascularization of human connective microtissues. Methods Mol Med 2007;140:153-66
- Krebs J, Ferguson SJ, Nuss K, et al. Sildenafil prevents cardiovascular changes after bone marrow fat embolization in sheep. Anesthesiology 2007; 107:75-81
- Krebs J, Ferguson SJ, Nuss K, Leskosek B, Hoerstrup SP, Goss BG, Shaw S, Aebli N. Plasma levels of endothelin-1 after a pulmonary embolism of bone marrow fat. Acta Anaesthesiol Scand 2007;51:1107-14
- Lehmann R, Zuellig RA, Kugelmeier P, Baenninger PB, Moritz W, Perren A, Clavien PA, Weber M, Spinas GA. Superiority of small islets in human islet transplantation. Diabetes 2007;56:594-603
- Lucchinetti E, Aguirre J, Feng J, Zhu M, Suter M, Spahn DR, Harter L, Zaugg M. Molecular evidence of late preconditioning after sevoflurane inhalation in healthy volunteers. Anesth Analg 2007;105:629-40
- Lucchinetti E, Ambrosio S, Aguirre J, Herrmann P, Harter L, Keel M, Meier T, Zaugg M. Sevoflurane inhalation at sedative concentrations provides endothelial protection against ischemia-reperfusion injury in humans. Anesthesiology 2007;106:262-8
- Moroni L, Curti M, Welti M, et al. Anatomical 3D fiber-deposited scaffolds for tissue engineering: designing a neotrachea. Tissue Eng 2007; 13:2483-2493
- Nocito A, Dahm F, Jochum W, Jang JH, Georgiev P, Bader M, Renner EL, Clavien PA. Serotonin mediates oxidative stress and mitochondrial toxicity in a murine model of nonalcoholic steatohepatitis. Gastroenterology 2007;133:608-18
- Nocito A, Georgiev P, Dahm F, Jochum W, Bader M, Graf R, Clavien PA. Platelets and platelet-derived serotonin promote tissue repair after normothermic hepatic ischemia in mice.
  Hepatology 2007;45:369-76
- Opitz I, Lardinois D, Arni S, Hillinger S, Vogt P, Odermatt B, Rousson V, Weder W. Local recurrence model of malignant pleural mesothelioma for investigation of intrapleural treatment.
  Eur J Cardiothorac Surg 2007;31:773-8
- Opitz I, Sigrist B, Hillinger S, Lardinois D, Stahel R, Weder W, Hopkins-Donaldson S. Taurolidine and povidone-iodine induce different types of cell death in malignant pleural mesothelioma.
  Lung Cancer 2007;56:327-36
- Qiang T, Hillinger S, Yang L, Korom S, Weder W. Trachea reconstruction using novel tissue engineered constructs. Swiss Med Wkly 2007;137 Suppl 155:90S-92S
- Schaser KD, Disch AC, Stover JF, Lauffer A, Bail HJ, Mittlmeier T. Prolonged superficial local cryotherapy attenuates microcirculatory impairment, regional inflammation, and muscle necrosis after closed soft tissue injury in rats. Am J Sports Med. 2007; 35(1):93-102

- Schmidt D, Achermann J, Odermatt B, Breymann C, Mol A, Genoni M, Zund G, Hoerstrup SP. Prenatally fabricated autologous human living heart valves based on amniotic fluid derived progenitor cells as single cell source. Circulation 2007;116:I64-70
- Schmidt D, Hoerstrup SP. Tissue engineered heart valves based on human cells. Swiss Med Wkly 2007; 137 Suppl 155:80S-85S
- Schmidt D, Mol A, Kelm JM, Hoerstrup SP. In vitro heart valve tissue engineering. Methods Mol Med 2007;140:319-30
- Schmidt D, Stock UA, Hoerstrup SP. Tissue engineering of heart valves using decellularized xenogeneic or polymeric starter matrices. Philos Trans R Soc Lond B Biol Sci 2007; 362:1505-1512
- Selzner M, Selzner N, Jochum W, Graf R, Clavien PA. Increased ischemic injury in old mouse liver: an ATP-dependent mechanism. Liver Transpl 2007;13:382-90
- Sun LK, Reding T, Bain M, Heikenwalder M, Bimmler D, Graf R. Prostaglandin E2 modulates TNF-{alpha}-induced MCP-1 synthesis in pancreatic acinar cells in a PKA-dependent manner. Am J Physiol Gastrointest Liver Physiol 2007;293:G1196-204
- Tan Q, Steiner R, Yang L, Welti M, Neuenschwander P, Hillinger S, Weder W. Accelerated angiogenesis by continuous medium flow with vascular endothelial growth factor inside tissue-engineered trachea. Eur J Cardiothorac Surg 2007;31:806-11
- Thomale UW, Bender M, Casalis P, et al. Tacrolimus depresses local immune cell infiltration but fails to reduce cortical contusion volume in brain-injured rats. Immunobiology 2007; 212:567-576
- Thomale UW, Bender M, Casalis P, Rupprecht S, Griebenow M, Neumann K, Woiciechowsky C, Unterberg AW, Stover JF. Tacrolimus depresses local immune cell infiltration but fails to reduce cortical contusion volume in brain-injured rats. Immunobiology 2007;212:567-76
- Von Känel R, Hepp U, Kraemer B, Traber R, Keel M, Mica L, Schnyder U. Evidence for low-grade systemic proinflammatory activity in patients with posttraumatic stress disorder. J Psychiat Res 2007; 41:744-52
- Zhai W, Cardell M, De Meester I, Augustyns K, Hillinger S, Inci I, Arni S, Jungraithmayr W, Scharpe S, Weder W, Korom S. Intragraft DPP IV inhibition attenuates post-transplant pulmonary ischemia/reperfusion injury after extended ischemia. J Heart Lung Transplant 2007;26:174-80

# 6. Grants 2007



**Cardiac Surgery**

| Grants | Title of Project | Project Leader |
|---|---|---|
| EU Grant Framework Program 6 (BioSys) | Intelligent Biomaterial Systems for Cardiovascular Tissue Repair | Prof. Zünd |
| Swiss National Science Foundation | Development of Cell-Based Therapies for Myocardial Defects | Prof. Hoerstrup |
| Hartmann-Müller-Stiftung | Prenatal Human Progenitor Cells for Tissue Engineering of Living Autologous Pediatric Cardiovascular Replacements | Dr. D. Schmidt<br>Prof. Hoerstrup |
| Novartis Stiftung | Tissue Engineering Heart Valves | Prof. Zünd<br>Prof. Hoerstrup |
| Bundesministerium für Bildung und Forschung (BMBF Grant) | Cryopreserved Umbilical Cord Cells For Heart Valves Tissue Engineering | Prof. Hoerstrup |
| Zurich Center for Integrative Human Physiology Grant 2006 – 2008 | Vulnerable atherosclerotic plaques - early detection, functional imaging, targeted treatment | Prof. Hoerstrup |
| National Competence Center of Research | Klinik für Herz- und Gefässchirurgie - Lead Cardiac Robotic Surgery Switzerland | Prof. Zünd |
| CO-ME | Robotics in Cardiovascular surgery | Prof. Zünd<br>PD Dr. Grünenfelder |
| Swiss Foundation for Research on Muscle Diseases | "M-band alterations characterize muscle pathogenesis" | I. Agarkova |
| Wolfermann-Nägeli-Foundation | "The role of M-band in the striated muscle sarcomere" | I. Agarkova |

**Visceral & Transplant Surgery**

| Grants | Title of Project | Project Leader |
|---|---|---|
| **Hepatobiliary laboratory** | | |
| SNF | Small-for-size liver transplantation: platelets and platelet-derived serotonin in the ischemic and regenerating liver | Prof. P.A. Clavien |
| SNF | In vivo analysis of liver regeneration and tissue repair in rodents using an animal MR | Prof. P.A. Clavien |
| SNF | Hypothermic oxygenated perfusion extracorporeal of the rat liver in non heart beating donors after cold storage | PD Dr. P. Dutkowski |
| Edoardo R., Giovanni, Giuseppe und Chiarina Sassella-Stiftung | Ceramidbasierte Behandlung des kolorektalen Karzinoms | Dr. F. Dahm/ Prof. P.A. Clavien |
| Roche Organ Transplantation Research | Protective Mechanisms of Pentoxyfilline for Liver Surgery and Liver Transplantation | PD. Dr. H. Petrowsky/ Prof. P.A. Clavien |
| UBS-Grant | Leukocytes and Thrombocytes Induce Liver Regeneration after Major Tissue Loss | Prof. P.A. Clavien |
| EMDO | Wirkung und Mechanismus der pharmakologischen Präkonditionierung bei zirrhotischer Leber | Dr. J.-H. Jang / Prof. P.A. Clavien |
| Hartmann Müller Stiftung EMDO | Die Rolle von Serotonin bei der Magensäuresekretion in humanen Magendrüsen | Dr. Ph.Kirchhoff |
| 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 |
| **Pancreatitis laboratory** | | |
| SNF | The role of COX-2 in chronic pancreatic inflammation and fibrosis | PD Dr. R. Graf |
| Velux Stiftung | The role of macrophages in chronic pancreatic inflammation | PD Dr. R. Graf |
| Amelie Waring Stiftung | Chronische Pankreatitis | PD Dr. R. Graf |
| **Islet-Transplantation laboratory** | | |
| Olga Mayenfisch | Präkonditionierung | Dr. W. Moritz |
| UBS-Grant | Der Einfluss des Microenvironments auf die Differenzierung von Stammzellen | Dr. P. Kugelmeier |
| Theodor und Ida Herzog-Egli Stiftung | The impact of the microenvironment on the differentiation of stem cells | Dr. P. Kugelmeier |

**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 wissenschaftliche Forschung der Universität Zürich | Nichterythroide Wirkungen von humanem rekombinaten Erythropoietin in der Traumatologie und rekonstruktiven Chirurgie der Extremitäten | Dr. Wanner |

**Plastic Hand & Reconstructive Surgery**

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

**Thoracic Surgery**

| Grants | Title of Project | Project Leader |
|---|---|---|
| Krebsliga | Pharmacokinetic Study after Intrapleural Topical Application of Chemotherapeutic Agent for malignant Pleuramesothelioma in Immune Competent Rat Model | PD Dr. Lardinois |
| Hartmann Müller-Stiftung | Pharmacokinetic Study after Intrapleural Topical Application of Chemotherapeutic Agent for malignant Pleuramesothelioma in Immune Competent Rat Model | PD Dr. Lardinois |
| Olga Mayenfisch-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 |
| Krebsliga | Prognostic markers for malignant pleural mesothelioma | Dr. Schmitt-Opitz |
| Krebsliga Zürich | Adjuvante intrapleurale Spüllösung nach Pleuro-pneumonektomie beim malignen Pleuramesotheliom | Dr. Schmitt-Opitz |
| Hartmann-Müller-Stiftung | The effect of NSAIDs on early inflammatory response after mechanical pleurodesis in a pig model | Dr. Schmitt-Opitz |
| Fellowship European Society of Medical Oncology | Intrapleural therapy after surgery for malignant pleural mesothelioma | Dr. Schmitt-Opitz |
| Olga Mayenfisch-Stiftung | Tissue-engineering zur Trachealrekonstruktion | PD Dr. Hillinger |
| Krebsliga | Epstein Barr virus-induced molecule 1 ligand chemokine in lung cancer therapy | PD Dr. Hillinger |
| Sassella-Stiftung | Epstein Barr virus-induced molecule 1 ligand chemokine (ELC⁄CCL19) | PD Dr. Hillinger |
| SNF | Immune targeted therapy for lung cancer | PD Dr. Hillinger |
| EMDO-Stiftung | Entwicklung eines in-vivo-Bioreaktors zur Reepithelial-isierung einer tissue-engineerten Neo-Trachea | PD Dr. Hillinger |
| Deutsche Forschungs-gemeinschaft | Entwicklung eines Modells der chronischen Abstossung nach Lungentransplantation | Dr. Jungraithmayr |
| SNF | Trachea reconstruction using novel tissue engineered constructs | Prof. W. Weder |
| Karitative Stiftung Dr. Gerber-ten Bosch, Zürich | - „Erweiterung des Organspendeangebotes von Lungentransplantationen“ LuTPL Pig | I.Inci |

**Urological Research**

| Grants | Title of Project | Project Leader |
|---|---|---|
| Forschungskredit der Universität Zürich | „Characterization of CTL immune activity against p53-binding regions of BKV large T antigen in BKV seropositive prostate cancer patients“ | Dr. M. Provenzano |

**Cranio-Maxillofacial Surgery Research**

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

**Surgical Intensive Care Medicine**

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

# 7. Awards 2007



- Dr. med. A. El Badry received the SGC - award at the annual meeting of the Swiss Society of Surgery
- PD Dr. med. H. Petrowsky received the SGC - award at the annual meeting of the Swiss Society of Surgery
- Dr. K. Furrer received the Research award from the Association of Research in Surgery (Swiss Society of Surgery)
- M. Lesurtel received the Swiss Transplant Research award at the annual Swiss Transplant meeting
- Dr. med. Isabelle Opitz received the Brompton Award from the European Association of Cardiovascular and Thoracic Surgery, Leuven/Belgium 09/07

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

Sponsors:



**AOTEC AG**