2012 Core-to-Core Summer Seminar for Young Researchers

20th & 21st August 2012
Seminar Room, Hans-Borst-Center
LEBAO, Hannover Medical School

Program

Monday, August 20th

Get Together at 8:50 a.m.

09:00 – 09:20 Opening and welcome greeting
Axel Haverich

09:20 – 09:40 Invitation speech and recent results in Hannover
Andres Hilfiker

09:40 -10:00 Study and Consortium updates
Masamichi Ono

10:00 - 10:30 Coffee Break

Part 1. Recent results of Clinical Trial for Myocardial regeneration

Hannover
10:30 - 11:00 Ingo Kutschka
Autologous Stem Cell Therapy for ischemic cardiomyopathy: current status

Osaka
11:00 - 11:30 Shigeru Miyagawa
Clinical application of Cell Sheet for heart failure

Helsinki
11:30 - 12:00 Ari Harjula
Bone Marrow Mononuclear Cell Transplantation combined with CAGB in Ischemic Heart Failure - A Placebo-controlled Randomized Double-Blinded Study

12:00 Lunch & Break

13:00 LEBAO visit

14:00 Excursion

18:00 Dinner

Tuesday, August 21st

Part 2. Special Session: TE Valve lectures

09:00-09:30 Serghei Cebotari (Basic Technology)
09:30-10:00 Thomas Breymann (Clinical Results)
10:00-10:30 Samir Sarikouch (ESPOIR Study)

10:30 -11:00 Coffee Break

11:00-11:30 Hideto Ozawa (Re OP for RVOT)
11:30-12:00 Axel Haverich (Summary)

12:00-13:00 Lunch
TE Valve meeting (Sawa, Harjula, Haverich)

Part 3. General Presentation

13:00-13:20 Sokichi Kamata
A Targeted Delivery of Adipocytokines into the Heart by Transplantation of Induced Adipocyte Cell-Sheet Yields Immune-tolerance and Functional Recovery in Autoimmune

13:20-13:40 Satoshi Kainuma
Combination of omentum implantation with cell sheet enhanced therapeutic effect in rat infarction model

13:40-14:00 Hassina Baraki
Intramyocardial Transplantation of Bioartificial Cardiac Tissue Splints as an interim step towards prevascularized bioartificial cardiac tissue?

14:00 -14:15 Coffee Break

14:15-14:35 Yukiko Imanishi
Cellular-based immune suppression in the allogenic skeletal myoblast sheet transplantation

14:35-14:55 Esko Kankuri
Stem cells ahead - proceed with caution

14:55-15:00 Ari Harjula
Closing remarks

Axel Haverich, MD, PhD.

Professor and Director,
Dept. of Cardiac, Thoracic,
Transplantation, and Vascular Surgery
Hannover Medical School
Hannover, Germany

Greeting from the German Coordinator

Dear colleagues, I have the privilege to welcome you to the next summer meeting of our international consortium for cardiac tissue engineering by JSPS´s Core-to-Core Program, Rebirth program of Deutsche Forschungsgemeinschaft (DFG) and bilateral program of Academy of Finland (AF).

After our previous meetings in Vienna, Geneva, Tampere, Nagoya, and Yokohama, we will for sure have extended results and innovations to report at our meeting in Hannover. I am very delighted, that so many of you have been able to participate this year.

The recent progress in cardiac tissue regeneration and patch technology will encourage us to further develop our research programs. Exchange of our students and post doc fellows makes real co-operation and understanding of our cultural and research as well as clinical practices even more realistic.

During next two days, I believe, we are all more convinced about the fruitfulness of our co-operation and continuation of the program in future.

I wish you all by heart welcome to the Hannover meeting. Please, enjoy also the clean air and beautiful nature of northern Germany.

Andres Hilfiker, PhD.

Principal Investigator,
Dept. of Cardiac, Thoracic,
Transplantation, and Vascular Surgery,
The Leibniz Research Laboratories for
Biotechnology and Artificial Organs
(LEBAO),
Hannover Medical School
Hannover, Germany

Welcome address and recent results in Hannover

It is a pleasure and honor to welcome you here at the Hannover Medical School. The next two days we will have the opportunity to exchange knowledge and progress in cardiovascular regeneration therapies from the very basic research up to clinical trials. For this, we are located so to speak in the “heart” of the excellence cluster “REBIRTH” at the Hans-Borst-Center.

I hope that beside the scientific value of this meeting, you will also have the chance to learn a little bit of Hannover, the capital of Lower Saxony, in respect to history and modern appearance. In the afternoon there will be a tour to show you some interesting places in Hannover.

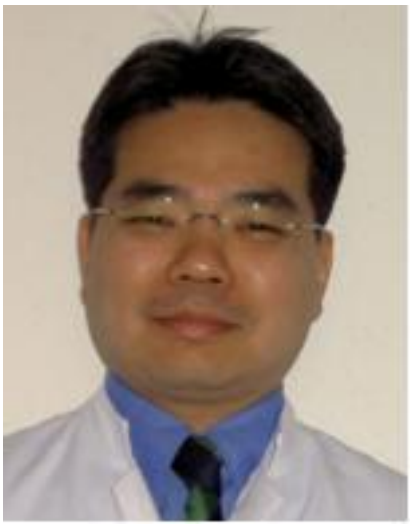
Where are we, and where are we going to in preclinical experimental cardiovascular TE? Concerning translational aspects of your findings in the last years in respect of heart valve replacement therapies you will be informed by presentations of Drs. Cebotari, Breymann and Sarikouch. Concerning myocardial tissue replacement therapies Dr. Baraki will present you a therapeutic approach. All talks schedule for tomorrow.

I just would like to present some new data concerning TE of heart valves in the elderly organisms (sheep), an aspect of major importance considering patients with aortic valve disease (average age of 65 years) as potential recipients of decellularized heart valve matrices.

Another major aspect is the limited availability of human heart valves for decellularization. The development of non-antigenic xenogenic heart valve matrices would solve this problem. A short overview of our current approach for a decellularized porcine non-antigenic xenograft might give an idea how this problem can be addressed.

Masamichi Ono, MD, PhD.

Principal Investigator,
Dept. of Cardiac, Thoracic,
Transplantation, and Vascular Surgery
Hannover Medical School
Hannover, Germany

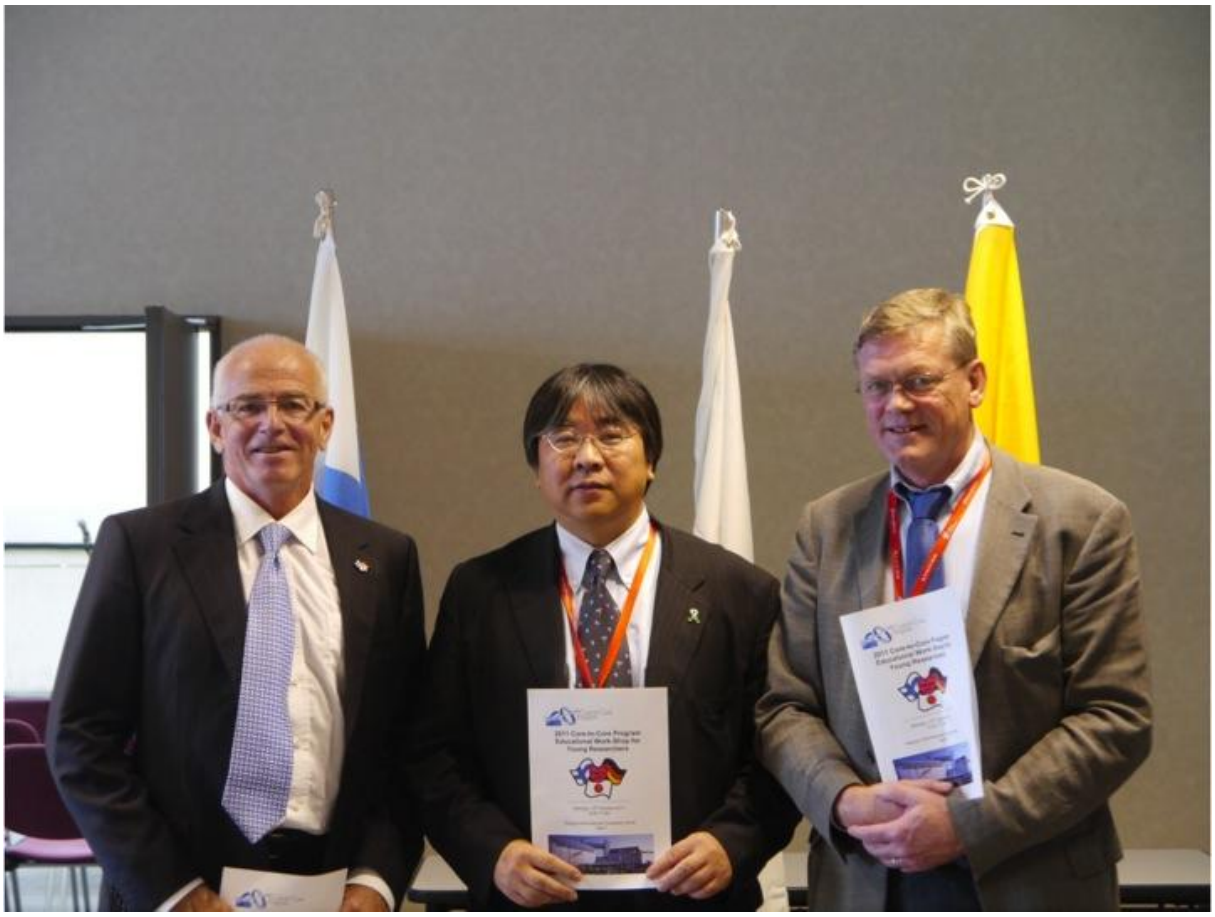


Study and Consortium updates

It is my great pleasure to welcome you to the 6th meeting of Core-to-Core Program at Hannover Medical School, the German Core Institute. Since 2011, Core-to- Core Program has been up-graded as Type A (Strategic Research Networks). In the coming 3 years, we can expand and strengthen research networks that will build strategic interdisciplinary research hubs in centered Japan, establish sustainable research partnerships between research institutions in Japan and other scientifically advanced countries, and contribute to fostering young researchers who will advance the next generation of science. Up to 30 million yen will be granted in each year, and the more active research works are expected including summer seminar for young researchers and international symposiums.

Adding to this meeting in Hannover, an international symposium and seminar for the young researchers have been performed on 12th and 13th June in Yokohama, during the 11th annual meeting of the Japanese society for Regenerative Medicine, which Prof. Sawa was the congress president. It is quite new program in the scheme of Core-to-Core Type A. Prof. Ari Harjula, and Prof. Axel Haverich are invited to the symposium and some lecturers from inside and outside of Japan are also performed.

I hope that fruitful discussion will be made in this meeting, and further development of our research consortium will be done in the coming years to be a real international research hubs in the field of cardiac tissue engineering and its clinical application.



2011.10.10
International workshop for young researchers
Nagoya International Congress Center
Annual meeting for Japanese society for Thoracic Surgery

Part 1.

Recent results of Clinical Trial for Myocardial regeneration

Ingo Kutschka, MD, PhD.

Dept. of Cardiac, Thoracic,
Transplantation, and Vascular Surgery
Hannover Medical School
Hannover, Germany

Autologous Stem Cell Therapy for ischemic cardiomyopathy: current status

To be updated.

Shigeru Miyagawa, MD, PhD.

Dept. of Cardiovascular Surgery
Osaka University
Osaka, Japan

Clinical application of Cell Sheet for heart failure

LVAD implantation and Heart transplantation have been well accepted as the ultimate lifesaving means of supporting end-staged heart failure patients. However, due to the limited durability of the LVAD and the shortage of donors, there are some limitations in these procedures. In this clinical situation, we developed cell sheet technology experimentally and introduced this to the treatment of severely damaged myocardium in clinical setting as translational research.

In a series of pre-clinical experiments using animal heart failure model, we proved that myoblast sheets could heal the impaired heart mainly by cytokine paracrine effect. Evidenced by these pre-clinical experiments, we applied myoblast sheets to 4 DCM patient receiving LVAD and 2 patients showed the recovery from LVAD. And we implanted 13 patients (ICM 8, DCM 5) without LVAD and some patients showed LV reverse remodeling.

Recently we were succeeded in the development of cardiomyocyte sheets derived from iPS cells, which showed functional recovery in porcine MI model.

Cell sheet technology may be a promising armamentarium for healing severely damaged myocardium.

Ari Harjula, MD, PhD.

Professor and Director,
Dept. of Cardiothoracic Surgery
University of Helsinki
Helsinki, Finland

Bone Marrow Mononuclear Cell Transplantation combined with CAGB in Ischemic Heart Failure -A Placebo-controlled Randomized Double-Blinded Study

Aims: Clinical studies suggest that intra-myocardial injection of bone marrow cells in conjunction with coronary artery bypass surgery improves left ventricular parameters in ischemic heart disease. However, there is a lack of properly conducted clinical trial.
Methods and Results: Forty patients with coronary disease and left ventricular ejection fraction (EF) ≤45% despite maximal medical treatment were randomized in a double-blinded manner to receive either bone-marrow cells ($9.1x\ 10^8 \pm 3.8x10^8$), or plain serum during coronary revascularization operation. Cardiac magnetic resonance imaging (MRI), positron emission imaging with fludeoxyglucose (PET) and single photon emission computed tomography (SPECT) was performed preoperatively to assess nonviable scar and 12 months after the operation the examinations were repeated. There was no mortality. One year control imaging was performed in 18 patients in each group. The EF improved from 39.6±9% to 43.6±9% in the treatment group and from 37.2±9% to 42.3±8% in the control group. There was no difference between the groups. Scar area assessed by MRI T2 late enhancement was measured 21.3±5% preoperatively and 19.8±6% at one year after the operation in the treatment group and 17.8±10% and 19.3±9% in the control group, respectively (p=ns). Percentual LV wall thickening at the site of the injections in the peri-infarct area was 33,5±27% preoperatively and 41,8±38% (p=ns) in the treatment group and 33,3±25% and 46,6±29% (p=ns) in the control group, respectively. There was no difference between the groups.
Conclusion: Modern heart failure medication combined with coronary bypass operation improves left ventricular function significantly. Intramyocardial injection of bone marrow cells has no measurable effects in the heart.

Part 2.

Special Session:
Tissue Engineered Valve
From bench to bedside and future prospective

Serghei Cebotari, MD.

Dept. of Cardiac, Thoracic,
Transplantation, and Vascular Surgery
Hannover Medical School
Hannover, Germany

Construction of autologous human heart valves based on an acellular allograft matrix.

OBJECTIVE:

Tissue engineered heart valves based on polymeric or xenogeneic matrices have several disadvantages, such as instability of biodegradable polymeric scaffolds, unknown transfer of animal related infectious diseases, and xenogeneic rejection patterns. To overcome these limitations we developed tissue engineered heart valves based on human matrices reseeded with autologous cells.

METHODS AND RESULTS:

Aortic (n=5) and pulmonary (n=6) human allografts were harvested from cadavers (6.2+/-3.1 hours after death) under sterile conditions. Homografts stored in Earle's Medium 199 enriched with 100 IU/mL Penicillin-Streptomycin for 2 to 28 days (mean 7.3+/-10.2 days) showed partially preserved cellular viability (MTT assay) and morphological integrity of the extracellular matrix (H-E staining). For decellularization, valves were treated with Trypsin/EDTA resulting in cell-free scaffolds (DNA-assay) with preserved extracellular matrix (confocal microscopy). Primary human venous endothelial cells (HEC) were cultivated and labeled with carboxy-fluorescein diacetate-succinimidyl ester in vitro. After recellularization under fluid conditions, EC were detected on the luminal surfaces of the matrix. They appeared as a monolayer of positively labeled cells for PECAM-1, VE-cadherin and Flk-1. Reseeded EC on the acellular allograft scaffold exhibited high metabolic activity (MTT assay).

CONCLUSIONS:

Earle's Medium 199 enriched with low concentration of antibiotics represents an excellent medium for long time preservation of extracellular matrix. After complete acellularization with Trypsin/EDTA, recellularization under shear stress conditions of the allogeneic scaffold results in the formation of a viable confluent HEC monolayer. These results represent a promising step toward the construction of autologous heart valves based on acellular human allograft matrix.

Thomas Breymann, MD.

Dept. of Cardiac, Thoracic,
Transplantation, and Vascular Surgery
Hannover Medical School
Hannover, Germany

Tissue engineered valve first clinical results

Degeneration of xenografts or homografts is a major cause for reoperation in young patients after pulmonary valve replacement. We present the early results of fresh decellularized pulmonary homografts (DPH) implantation compared with glutaraldehyde-fixed bovine jugular vein (BJV) and cryopreserved homografts (CH).
METHODS AND RESULTS:
Thirty-eight patients with DPH in pulmonary position were consecutively evaluated during the follow-up (up to 5 years) including medical examination, echocardiography, and MRI. These patients were matched according to age and pathology and compared with BJV (n=38) and CH (n=38) recipients. In contrast to BJV and CH groups, echocardiography revealed no increase of transvalvular gradient, cusp thickening, or aneurysmatic dilatation in DPH patients. Over time, DPH valve annulus diameters converge toward normal z-values. Five-year freedom from explantation was 100% for DPH and 86 ± 8% and 88 ± 7% for BJV and CH conduits, respectively. Additionally, MRI investigations in 17 DPH patients with follow-up time >2 years were compared with MRI data of 20 BJV recipients. Both patient groups (DPH and BJV) were at comparable ages (mean, 12.7 ± 6.1 versus 13.0 ± 3.0 years) and have comparable follow-up time (3.7 ± 1.0 versus 2.7 ± 0.9 years). In DPH patients, the mean transvalvular gradient was significantly (P=0.001) lower (11 mm Hg) compared with the BJV group (23.2 mm Hg). Regurgitation fraction was 14 ± 3% and 4 ± 5% in DPH and BJV groups, respectively. In 3 DPH recipients, moderate regurgitation was documented after surgery and remained unchanged in follow-up.
CONCLUSIONS:
In contrast to conventional homografts and xenografts, decellularized fresh allograft valves showed improved freedom from explantation, provided low gradients in follow-up, and exhibited adaptive growth.

Samir Sarikouch, MD, PhD.

Dept. of Cardiac, Thoracic,
Transplantation, and Vascular Surgery
Hannover Medical School
Hannover, Germany

European clinical study for the application of regenerative heart valves (ESPOIR Study)

Aquired and congenital heart disease can necessitate heart valve replacement. However, none oft he currently available heart valve substitutes are considered as an idenal replacement as all lack the potential of growth. They require anticoagulation, bearing the rrisk of bleeding when manufactured from non-organic material, or degenerate where derived from animals or human tissue donors (homografts), leading tot he need for frequent reoperation, especially in children and younger patients.
The ESPOIR consortium (European clinical study for the application of regenerative heart valves) proposes a clinical trial fort he evaluation of a

- Newly develop regenerative heart valve (DHV)
- Based on the decellulatization of homografts by tissue engineering methods and autologous recellularization
- Which can overcome the significant limitations of current solutions, paving the way for an ideal valve substitute.

ESPOIR is based on auspicious early clinical results in children and young adults. In order to drive translation of this promising regenerative approach towards widespread practical clinical use and to reduce the burden of congenital heart defects, we propose a prospective trial which will include at least 200 patients from 8 leading European Centres for Congenital Cardiothoracic Surgery, enabling robust statistical analysis for exact scientific evaluation of this innovative new therapy.

The main objective of the proposed ESPOIR project is translation of the innovative regenerative therapy presented by the DHV regenerative heart valve into widespread clinical use. To achieve this goal, the ESPOIR consortium will:

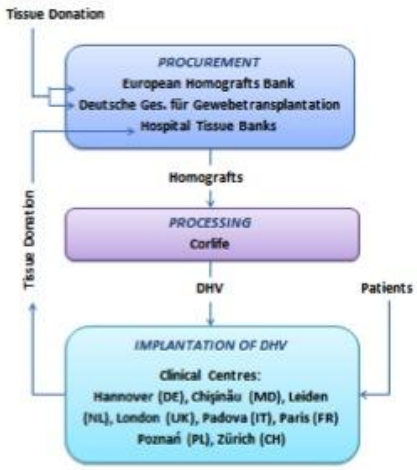
- Evaluate DHV for pulmonary valve replacement rates in comparison to current valve substitutes within a large prospective multicenter trial at 8 leading European Centres for Congenital Cardiothoracic Surgery regarding reoperation and re intervention, hemodynamic performance, growth potential, and long term durability.
- Establish sustained structures for European-wide homograft procurement with special emphasis on small homograft sizes.

- Disseminate the results of ESPOIR to the scientific community, patient organizations and political stakeholders
- Implement exploitation structures, e.g. partnership models of the decellularization technique for local, national or European homograft banks.

ESPOR will have a dramatic impact on the treatment of congenital, but also of acquired heart defects, as superior performance, growth potential and long term durability of these decellularized heart valves (DHV) can be confirmed within the proposed trial, allowing the rapid translation of this regenerative approach to clinical practice.

The ESPOR consortium

www.espoir-clinicaltrial.eu



Hideto Ozawa, MD.

Dept. of Cardiovascular Surgery
Osaka University
Osaka, Japan

Re-operation for right ventricle outflow tract with Bioprosthetic Valve: Osaka University Experience

Background:
Patients after reconstruction of right ventricle outflow tract (RVOT) have been revealed to be susceptible to progressive pulmonary valve regurgitation (PR). Development of novel method to quantify the right as well as left ventricular function, in addition to PR has clarified the deleterious effect of PR on both ventricles, resulting in lowering the threshold for pulmonary valve replacement (PVR). With this background in mind, we have performed PVR since 2000 on patients who have matched the following criteria: patients with symptoms of right heart dysfunction or those with right ventricle end-diastolic volume index of > 150(ml/m^2).
So as to analyze the mid-term result of our PVR method, we retrospectively detailed the qualitative as well as quantitative data of patients after PVR on RVOT lesion.
Operative method:
PVR was performed with beating heart condition under cardiopulmonary bypass. We have used Carpentier Edwards Perimount（CEP）Magna Valve or St Jude Medical Epic Valve for PVR (n=37). Also, in patients with extreme difficulty in implanting stented bioprosthetic valve, we have used stentless valve (PrimaPlus) in small number of patients (n=2).After removing the previous patch on RVOT, the bioprosthetic valve was situated at the site of pulmonary valve cusp, sutured with 4-0 prolene running suture. In case,RVOT is not wide enough to house the valve, woven Dacron graft patch was trimmed and used to accommodate the valve.
Results:
39 patients underwent PVR long term after the primary repair of congenital heart disease including tetralogy of Fallot, DORV, TGA and PA with VSD. Mean age at re-operation was 31(15～54) years old. All patients could be implanted bioprosthetic valve. With 8 years follow up; there were three patients who have to be replaced the valve because of valve insufficiency. Of those, two patients had been implanted a stent less valve (Prima Plus), and one patient stented valve (CEP valve).
In patients without reoperation on implanted bioprosthetic valve, maximal pressure gradient of the implanted valve were not increased significantly. Also, pulmonary regurgitation did not increased during the follow up period. With quantitative analysis of right / left ventricular function utilizing cardiac MRI, not only right ventricle function but left ventricle function has also been shown to improve accordingly.
Conclusions:
Durability of the stented bioprothetic pulmonary valve was satisfactory. Bioprothetic pulmonary valve replacement could have improved right as well as left ventricle function.

Part 3.

General Presentation

Sokichi Kamata, MD.

Dept. of Cardiovascular Surgery
Osaka University
Osaka, Japan

A Targeted Delivery of Adipocytokines into the Heart by Transplantation of Induced Adipocyte Cell-Sheet Yields Immune-tolerance and Functional Recovery in Autoimmune-Associated Myocarditis in Rat

Background: Clinical prognosis is critically poor in fulminant myocarditis, while its initiation or progression is fated in part by T cell-mediated autoimmunity. Adiponectin (APN) and associated adipokines were shown to be immune-tolerance inducers, though clinically-relevant delivery method into target pathologies is still under debate. We hypothesized cell sheet-based delivery system of adipokines might induce immune-tolerance and functional recovery in rat experimental autoimmune myocarditis (EAM).
Methods and Results: Scaffold-free induced adipocyte cell-sheet (iACS) was generated by differentiating syngeneic adipose tissue-derived stem cells into adipocytes on temperature-responsive dishes. Lewis rats with EAM, induced by immunization with myosin, underwent iACS implantation onto the anterior heart or sham operation. Supernatants of iACS contained a high level of APN and hepatocyte growth factor (HGF), and reduced proliferation of CD4-positive T cells that were sorted from splenocytes of the EAM rats *in-vitro*. Immunohistolabelling showed that CD4-positive T cells significantly less migrated in the anterior myocardium at 21 days after the iACS implantation than the sham. Consistently, the iACS implantation induced reduced cardiac expressions of IFNγ and IL17, and elevated level of APN and HGF compared to the sham. Consequently, left ventricular ejection fraction was significantly greater at 56 days after the iACS implantation (56.7±5.0%) than the sham (35.3±5.0%, $P<0.001$) in association with less collagen accumulation.
Conclusion: The targeted delivery of adipokines using tissue-engineered iACS ameliorated cardiac performance of the EAM rat model *via* effector T cell suppression, and induction of immune-tolerance. These findings might suggest a

potential of this tissue-engineered drug-delivery-system in treating fulminant myocarditis in the clinical settings.

Satoshi Kainuma, MD.

Dept. of Cardiovascular Surgery
Osaka University
Osaka, Japan

Effects of Pedicle Omentum Flap Combined With Cell Sheet Implantation on Vessel Stability and Left Ventricular Reverse Remodeling in Rat Myocardial Infarction Model

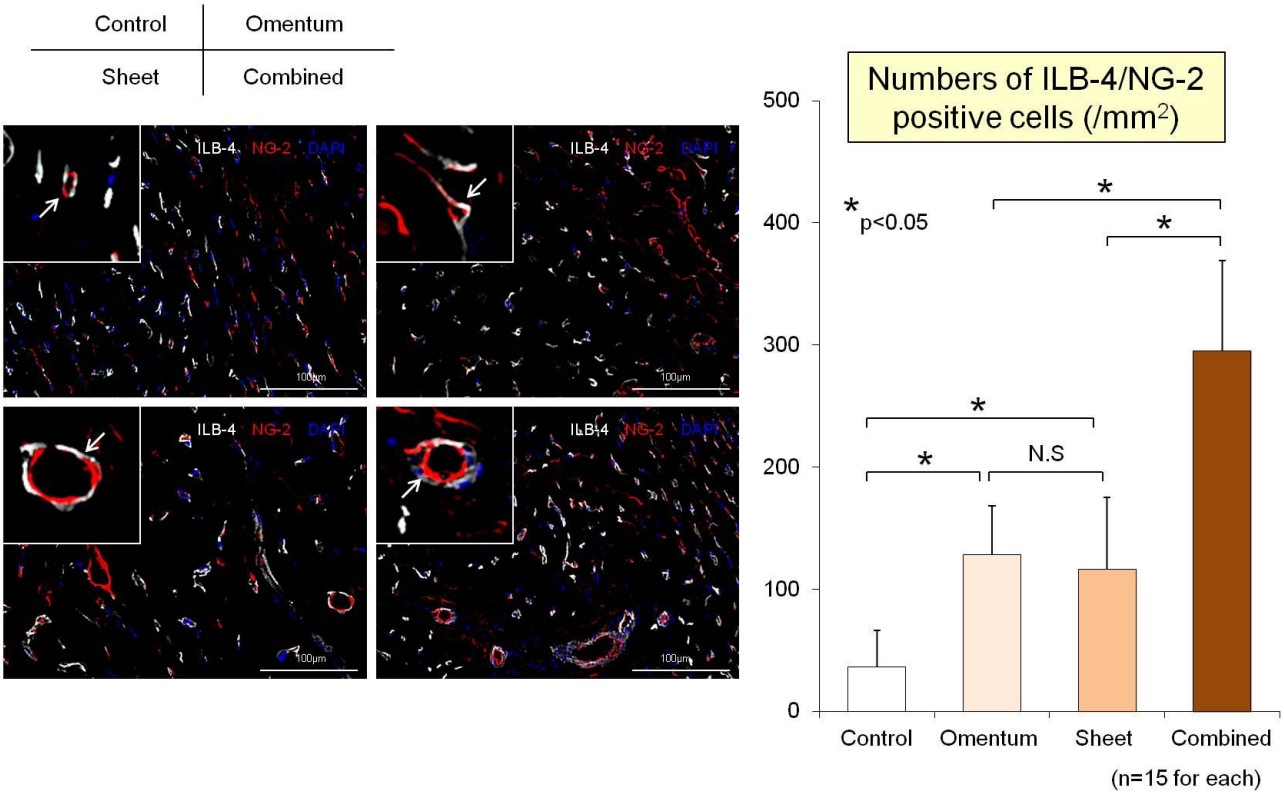
Objective: Cell sheet implantation has been shown to induce therapeutic angiogenesis in damaged myocardium, though instability of newly formed vessels may limit the effects. Accelerating maturation of new vessels to generate a well-organized vasculature may thus be crucial for producing therapeutic effects with this treatment. We speculated that a pedicle omentum flap combined with cell sheet therapy may modulate neovascular maturation, leading to enhanced functional recovery.

Methods: Cell sheets were constructed by culturing skeletal myoblasts of GFP-transgenic Lewis rats. Left coronary artery-ligated wild-type Lewis rats were divided into 4 groups; cell sheet transplantation (1.0×10^7 cells) plus omentum flap (combined), cell sheet alone (sheet), omentum alone (omentum), and sham operation (control) (n=15 for each).

Results: At 12 weeks after transplantation, combined group kept a large numbers of the GFP positive (donor) cells and recruited a large numbers of the c-kit$^+$ cells around the MI, as compared with the sheet group. The combined group also showed greater numbers of pericyte-lined neovascular endothelium (NG-2$^+$/ILB-4$^+$) in the peri-infarct myocardium as compared to the other groups **(Figure)**. Serial echocardiography indicated persistent improvement in LV function (reverse remodeling) in the

combined group (ejection fraction: 36±3 at baseline vs. 47±3 at 4 weeks vs. 51±4% at 12 weeks), but not in the other groups (sheet; 36±4 vs. 44±4 vs. 41±2%, omentum; 37±4 vs. 39±4 vs. 39±3%, control; 38±5 vs. 36±3 vs. 34±3%, respectively). Celiac artery angiography using india ink demonstrated communication between the pedicle omentum and native coronary arteries, which was also shown by histological analysis.

Conclusions: A pedicle omentum combined with cell sheet transplantation increased a well-organized and functional vasculature accompanied with a potent functional recovery in rat myocardial infarction model, suggesting a promising clinical application for failing heart.



Hassina Baraki, MD, PhD.

Dept. of Cardiac, Thoracic,
Transplantation, and Vascular Surgery
Hannover Medical School
Hannover, Germany

Intramyocardial Transplantation of Bioartificial Cardiac Tissue Splints as an interim step towards prevascularized bioartificial cardiac tissue?

Background: The delivery modality of bioartificial tissue grafts is one of the key limiting factors of myocardial restoration resulting in poor engraftment and survival of the transplanted tissue. Here, we present a new technique of intramyocardial application of solid bioartificial cardiac tissue (BCT) in a rat model.

Methods: BCTs derived from GFP-transgenic neonatal Lewis rat hearts were conditioned in a functional bioreactor for 14 days. In order to evaluate the impact of the surgical transplantation technique on cardiac function, myocardial infarction was induced (male, 8-10 weeks old, GFP -negative Lewis rats) by LAD ligation. Two weeks later, the GFP-positive BCTs were implanted in the infarcted area. The following groups are currently under investigation: (A) intramyocardial application of BCTs (n=10) versus (B) epicardial application (n=10) and (C) Sham group (myocardial infarct only). Cardiac function is analyzed by Magnetic resonance Imaging (MRI), echocardiography and conductance catheter 4 weeks after implantation. Conductance catheter examination and histological analyses are performed 4 weeks after implantation.

Results: We present the first histological and functional data of this ongoing study. Intramyocardially implanted BCTs can be identified by echocardiography und MRI (Fig.1). Fluorescence microscopy revealed that the implanted BCTs survive for 4 weeks. Neovascularization and perfusion could be identified inside the implanted BCTs (Fig.2). Epicardially transplanted BCTs did not engraft in the infarcted area and were divided from the recipient myocardium by a fibrous layer. Compared to controls BCT grafting led to a marked thickening of the infarcted myocardium in group A, resulting in an improvement of left heart function as shown by echocardiography, MRI and conductance catheter analyses. Histological and functional analyses of group B are still pending.

Conclusion: BCTs can be successfully implanted into infarcted myocardium of rats using a new needle transfer technique. The transplanted BCTs engraft and

vascularize in the infarcted area resulting in an improvement of left heart function. A combination of this application technique with iPS-cell technology may present a powerful strategy for myocardial restoration in ischemic cardiomyopathy

Yukiko Imanishi, PhD.

Exchanged Researcher,
Dept. of Cardiothoracic Surgery
University of Helsinki
Helsinki, Finland

Cell-based immunosuppression for allogenic skeletal myoblast sheet transplantation

Introduction

In the clinical study, we have proved that transplantation of autologous skeletal myoblast (SMB) sheet onto the heart is effective and safe for ischemic cardiomyopathy, whereas this method still has limitation for use in the emergency case. Transplantation of allogenic, instead of autologous, SMB sheet will be an optimized cell therapy for cardiac injury. Immunorejection is a major issue for allo-SMB therapy. Recently, mesenchymal stem cell (MSC) is reported to have immunosuppressive activity which is mediated by anti-inflammatory cytokines secretion such as induced nitric oxide synthase (iNOS). We hypothesized that by mixing MSCs with allo-SMB sheets, the immune responses induced by allo-SMB are reduced, thereby resulting in the attenuation of cardiac dysfunction after MI.

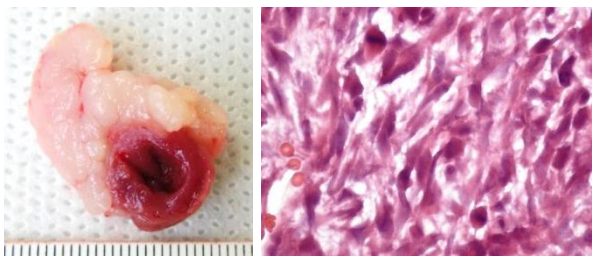
Methods and results

In vitro analysis, MSCs suppressed lymphocyte proliferation induced by allo-SMBs. iNOS expression was upregulated by combined stimulation of interferon gamma (IFNγ) and lipopolysaccharide (LPS). Cell sheets consisting of luciferase-expressing allo-SMB and MSC (mixed sheet) were transplanted subcutaneously to Wistar rats. Allo-SMBs retention was evaluated using bioluminescence imaging. Mixing MSCs prolonged allo-SMB retaention, which was enhanced by using stimulated MSCs. Next, we conducted mixed sheets transplantation using myocardial infarction model of rats. Four weeks after treatment, tumor growth was observed in mixed sheet received hearts. The tumor size and frequency were greater in the group which MSC was stimulated with IFNγ and LPS before creating cell sheet.

Conclusion

Our result suggests mixing MSCs may suppress immunoreaction and prolong the allo-SMB retention. However there is a possible risk that MSCs mixed with allo-SMBs induce tumor transformation, which is enhanced by inflammatory stimulation. Now we are planning to conduct a comprehensive analysis of gene expression in MSCs.

Figure. Tumor formation of the mixed sheet received heart



Esko Kankuri, MD, PhD.

Principal Investigator
Biomedicum Helsinki
University of Helsinki
Helsinki, Finland

Stem cells ahead - proceed with caution

The true therapeutic benefit of stem cell therapies has been investigated already for several decades. While this research has identified huge potential for regeneration of many tissues and organs, alternate reports indicate a darker side. Prompt therapeutic applications require the isolated and usually small population of stem cells to be aggressively propagated. Once cultured and stimulated to proliferate to be able to harvest enough cells for therapy in as short time as possible, the cells' suppressive control mechanisms may be compromised. Thus caution is called for when designing and conducting stem cell therapies. The benefits and risks of mesenchymal stem cells are discussed.

Participants:

From Osaka:

Sawa Yoshiki
Ueno Takayoshi
Miyagawa Shigeru
Nishi Hiroyuki
Saito Atsuhiro
Kamata Sokichi
Kainuma Satoshi
Ozawa Hideto
Kawamura Takuji
Shibamoto Ai

From Helsinki:

Harjula Ari
Kankuri Esko
Imanishi Yukiko

From Hannover:

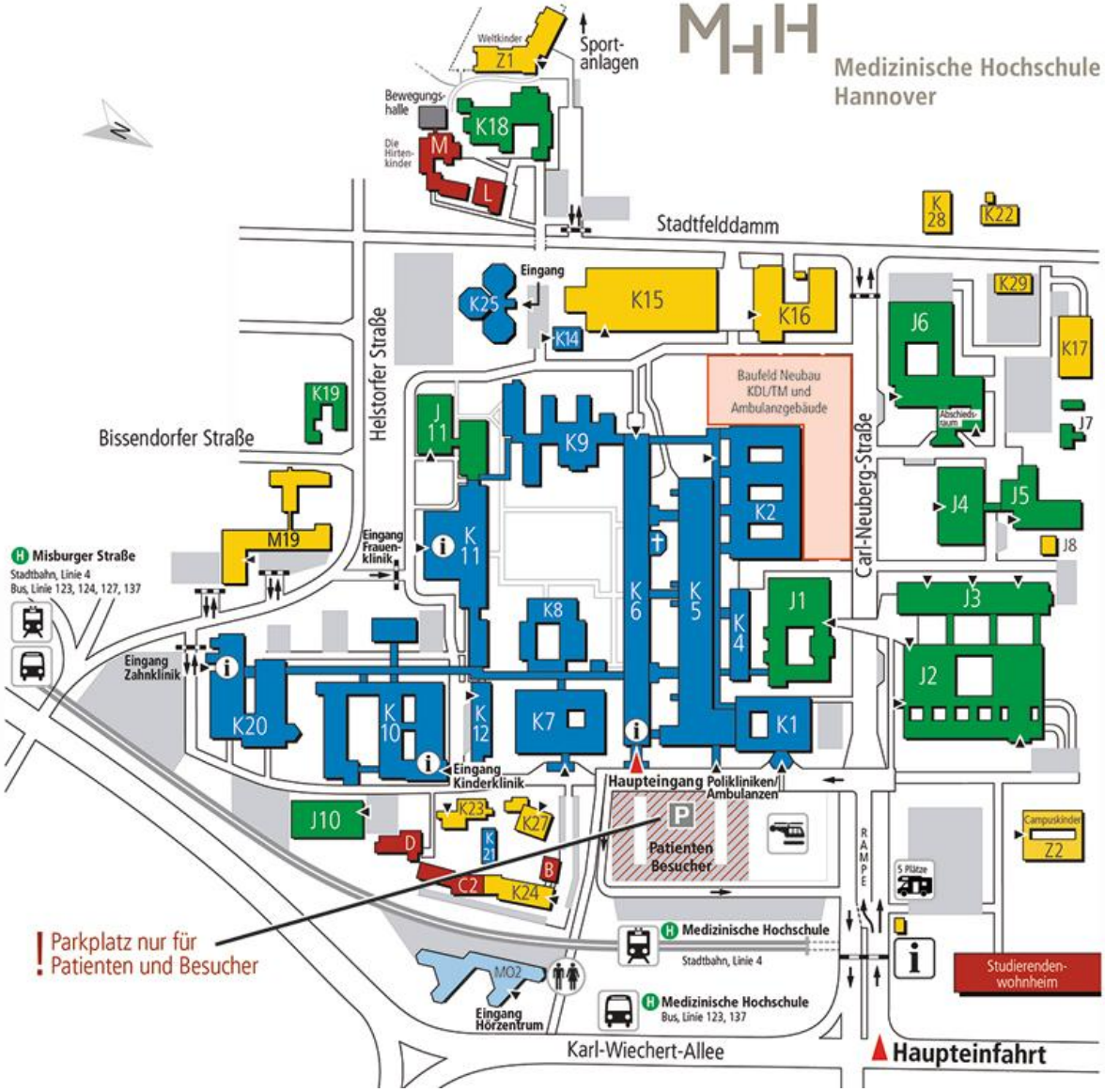
Haverich Axel
Baraki Hassina
Hilfiker Andres
Kutschka Ingo
Ono Masamichi
Saito Shunsuke
Gruh Ina
Cebotari Serghei
Breymann Thomas
Horke Alexander
Sarikouch Samir
Zweigerdt Robert
Lux Marco
Kensah George

Venue

The venue is located in the Campas MHH.

Hans-Borst-Center (J11)

Hannover Medical School,
Carl-Neuberg-Str.1
30625 Hannover, Germany
http://www.lebao.de/



Core Institution Address

Dept. of Cardiovascular Surgery
Osaka University Graduate School of Medicine
Yamada-oka 2-2, Suita, 565 0871, Osaka, Japan
Telefon: 81-6-6879-3154
Miss. Atsuko Shimai

Dept. of Cardiac, Thoracic, Transplantation and Vascular Surgery
Hannover Medical School
Carl-Neuberg Stra.1, 30625 Hannover, Germany
Telefon: 49-511-532-9397
Masamichi Ono. MD.
E-Mail: ono.masamichi@mh-hannover.de

Dept. of Cardiothoracic Surgery
University of Helsinki, Meilahti Hospital
Haartmaninkatu 4, 00029, Helsinki, Finland
Telefon: 358-9-4717-2305
Mrs. Kaisu-Maarit Pelkonen

Please refer to the following web site

http://jsps-osaka-u.jpn.org/