Progress Meeting
on
International consortium
for cardiac tissue engineering

August 22-24, 2011

Hurman Wanha Kansakoulu
Tampere, Finland

Core Institutions

Osaka University Graduate school of Medicine
Department of Cardiovascular Surgery
Head: Prof. Yoshiki Sawa

University of Helsinki
Department of Cardiothoracic Surgery
Head: Prof. Ari Harjula

Hannover Medical School
Department of Cardiac, Thoracic, Transplantation, and Vascular Surgery
Head: Prof. Axel Haverich

Co- Institutions

University of Tampere (Finland)
University of Oulu (Finland)
University of Rostock (Germany)
University of Düsseldorf (Germany)
Queen Mary University of London (UK)
University of Gent (Belgium)

Support Organizations

Core-to-Core Program
Japanese Society for Promotion of Science (JSPS)

Bilateral Program
Academy of Finland (AF)

Rebirth Program
Deutsche Forschungsgemeinschaft (DFG)

Core-to-Core Program Annual Meeting 2011, August 22nd – 24th

Venue: ***Hurman Wanha Kansakoulu (Hurma Old Elementary School), Tampere***
http://www.hurmankoulu.com/

The venue is located about 20 min. from the Tampere City.

Monday, August 22nd

Bus transportation starting from Hotel Ilves at 8.30 a.m.

If you are going to swim or to take a sauna at Ari Harjula's summer house, please take the towel from the hotel with you.

09.00 – 09.05 Opening and welcome – **Ari Harjula**

09.05 – 09.20 Invitation speech – **Pekka Heikkinen**

09.20 -11.00 Study updates

Esko Kankuri
Masamichi Ono
Andres Hilfiker

11.00 ***Lunch***

12.30 Transportation to Ari Harjula's summer house

Tuesday, August 23rd

Bus transportation starting from Hotel Ilves at 9.30 a.m.

Presentation 20 min, discussion 10 min

10.00-10.30 Myoblast sheet implantation can prevent the impairment of cardiac diastolic function after ventricular restoration by modulating the extracellular matrix gene expression
- **Shunsuke Saito**

10.30-11.00 Novel regenerative therapy using cell-sheet covered with omentum flap enables to deliver a huge number of cells in a porcine myocardial infarction model
- **Yasuhiro Shudo**

11.00-11.30 Induced pluripotent stem cell-derived cardiomyocytes form electrical coupling with native
myocardium contributing to functional recovery in rat chronic infarction model
- **Takahiro Higuchi**

11.30 -12.30 ***Lunch***

12.30-13.00 Combined CABG and Stem-Cell Transplantation for Heart Failure
- **Tommi Pätilä**

13.00-13.30 Myocardial cell retention and biodistribution after cardiac cell transplantation
- **Ingo Kutschka**

13.30-14.00 Effect of bone marrow stromal cell-sheet transplantation for myocardial infarction in rat - A comparison with skeletal myoblast sheet
- **Yukiko Imanishi**

14.00-14.30 ***Coffee break***

14.30-15.00 Intramyocardial transplantation of bioartificial cardiac tissue splints for restoration of infarcted myocardium in rats
- **Hassina Baraki**

15.00-15.30 Preliminary studies with Vineberg procedure in rat myocardium
- **Outi Villet**

15.30-16.00 Everolimus enhances allogeneic myoblast sheet therapy in rat acute myocardial infarction
- **Antti Siltanen**

16.00-17.00 Conclusion

17.00 ***Dinner***

Wednesday, August 24th Departures

Participants:

From Osaka:

Higuchi Takahiro
Imanishi Yukiko
Miyagawa Shigeru
Nishi Hiroyuki
Saito Atsuhiro
Saito Shunsuke
Shudo Yasuhiro

From Hannover:

Baraki Hassina
Hilfiker Andres
Kutschka Ingo
Ono Masamichi
Saito Tetsuya

From Helsinki:

Harjula Ari
Hämmäinen Pekka
Kankuri Esko
Pätilä Tommi
Siltanen Antti
Vento Antti
Villet Outi

Greeting from Finnish coordinator

Dear colleagues,
I have the privilege to welcome you to the next Progress 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 and Geneva we will for sure have extended results and innovations to report in Tampere meeting. I am very delighted, that so many of you have been able to participate our this year communications.

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 these two day sessions I believe, we are more convinced about the fruitfulness of our co-operation and continuation of the program in future.

I wish you all by heart welcome toTampere meeting. Please, enjoy also the clean air and beautiful nature of southern Finland.

Ari Harjula
Professor and chairman
Department of Cardiothoracic Surgery
Helsinki University Graduate School of Medicine

Abstract

With the autumn 2011 - spring 2012 marking the ending of many funding periods for the Helsinki team, we are looking forward to more productive years together with the Core Collaboration. The work thus far in the Consortium has been fruitful and we have gained much expertise and knowledge. New ideas have emerged, new grant applications have been filed, and there are still dead lines to catch. We are also very happy to announce collaboration with the Finnish Red Cross Blood Service. With some of the new applications approved and with the student exchange active, we are hoping the coming years are again workful and productive.

Esko Kankuri, Principal investigator (Helsinki)

Core-to-Core Type A (Strategic Research Networks). -future plan in the new era-

It is my great pleasure to hold the third meeting of Core-to-Core Program at Tampere in Finland, following the last meeting in Geneva. During the year, 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 Tampere, a workshop for the young researchers is planed on 10th October in Nagoya, during the 64th annual meeting of the Japanese society for Thoracic surgery. The title of the workshop is “Present and future scope of myocardial regeneration”. 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 workshop and some lecturers from inside and outside of Japan are also invited.

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.

Masamichi Ono, MD, PhD.
Dept. of Cardiac, Thoracic, Transplantation, and Vascular Surgery
Hannover Medical School
Hannover, Germany

2-2. Recent research achievements in Hannover

Re-endothelialization of BioVaM/SIS, suitable carrier matrices for myocardial Tissue Engineering

Andres Hilfiker

Background

Tissue engineering of bioartificial cardiac tissue represents a promising method for the repair of ischemic heart tissue after cardiac infarction. However, a persistent problem with the generation of thicker 3D tissues for therapeutic application is efficient nutrient supply and waste product removal in complex constructs. Thus, our *in vitro* approach combines 1) the re-endothelialization of a biological vascularized matrix (BioVaM) as a means to supply oxygen and nutrients, 2) construction of a thick, durable cardiac construct on top of the BioVaM; and 3) mechanical and electrical stimulation of the construct with a custom designed Bioreactor. Presented here in brief and in the seminar to be given is a summary of our current status and ongoing progress.

Methods

Decellularized porcine small intestinal segments with preserved arterial and venous pedicles (BioVaM), have been re-endothelialized with stably transfected rat endothelial cells RHE expressing GFP or RFP, porcine derived endothelial-like cells (pEC-like) or alternatively repopulated with transfected porcine smooth muscle cells (SMC) or BM-MSC. Thicker gel constructs generated by the use of iolated neonatal cardiomyocytes (CM) were histologically analyzed. Culture parameters including co-culture conditions, number of cells and cultivation time were investigated. BioVaM and/or cardiac constructs were investigated histologically and functionally where appropriate.

Results

Repopulation experiments of the decellularized matrix revealed fine and distinct structures for the arterial vessel bed, whereas the venous vessel bed is more susceptible to mechanical/chemical stress of decellularization. However, successful reendothelialization of arterial and venous vessel bed (Figure 1) was achieved after 14 days of static cultivation of the matrix. Perfusion of the matrix was beneficial for the repopulation of big vessel structures. In perspective to the reconstruction of whole vessels porcine SMC and bone marrow derived MSC were used for repopulation studies as well. In parallel co-culture experiments with SMC and EC were conducted. The construction of SIS - gel constructs revealed an aligned and uniform contractile unit when seeded on top of BioVaM. Endothelial cells co-isolated with CM or externally added formed net structures within the constructs (Figure 2).

Conclusions

Intermediate steps towards achieving our goal of an *in vitro* myocardial patch have been accomplished. Utilization of all tools established, as well as implementation of a new Bioreactor with our current constructs will support continued progress.

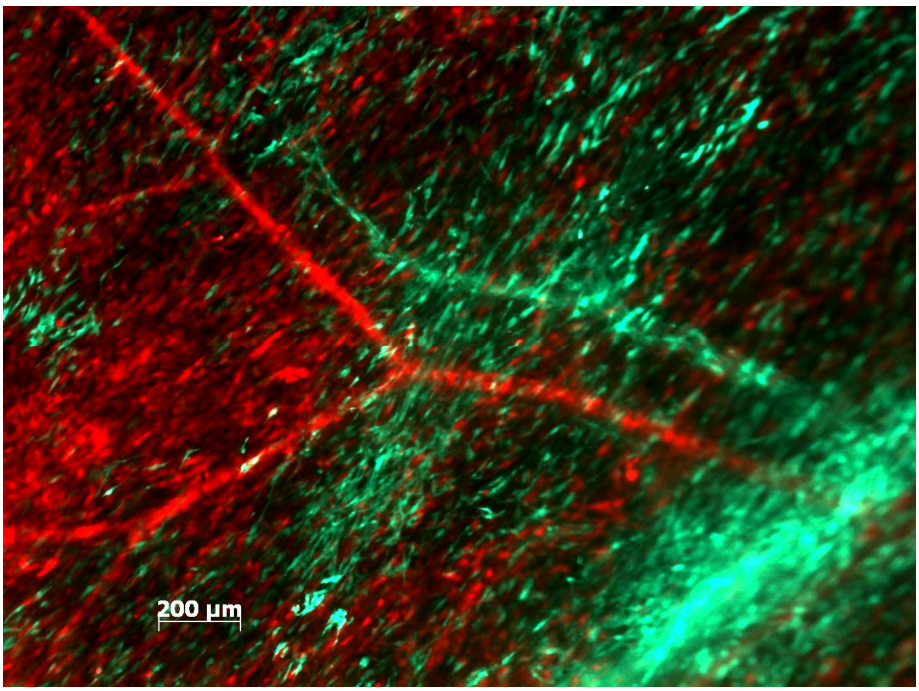


Figure 1: BioVaM reseeded with GFP expressing RHE (veins) and RFP expressing RHE (arteries).

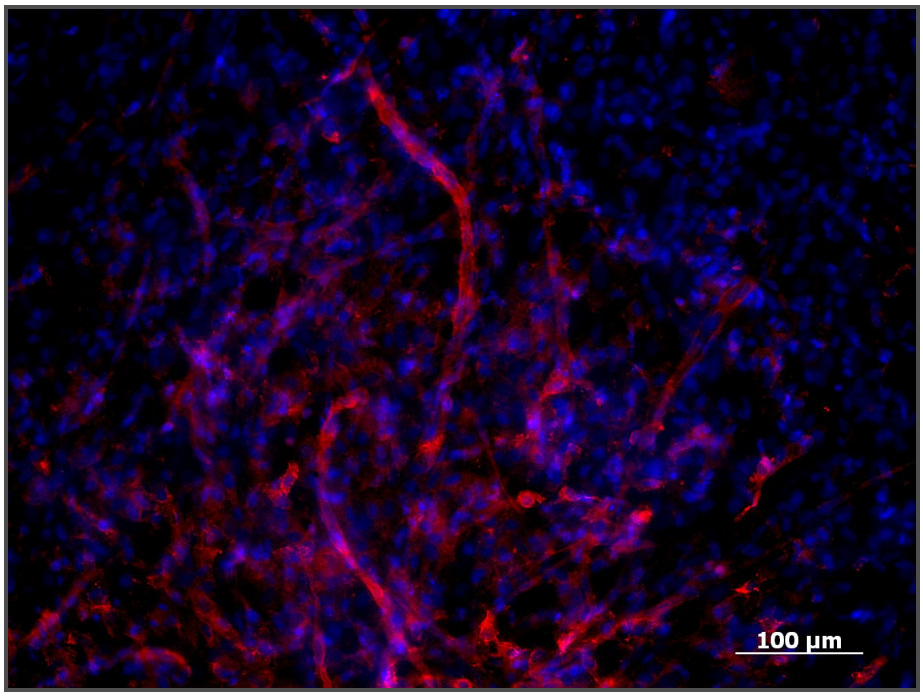


Figure 2: Net structures formed by endothelial cells in gel construct.

Myoblast Sheet can Prevent the Impairment of Cardiac Diastolic Function and Late Remodeling after Left Ventricular Restoration in Ischemic Cardiomyopathy

Shunsuke Saito, Taichi Sakaguchi, Shigeru Miyagawa, Hiroyuki Nishi, Yasushi Yoshikawa, Satsuki Fukushima, Atsuhiro Saito, Yasuhiro Shudo, Takahiro Higuchi, Yukiko Imanishi, and Yoshiki Sawa

Department of Cardiovascular Surgery, Osaka University Graduate School of Medicine, Suita, Osaka, Japan

Abstract

[Background]

Impairment of diastolic function and late remodeling are concerns after left ventricular restoration (LVR) for ischemic cardiomyopathy. This study aims to evaluate the effects of combined surgery of myoblast sheets (MS) implantation and LVR.

[Methods]

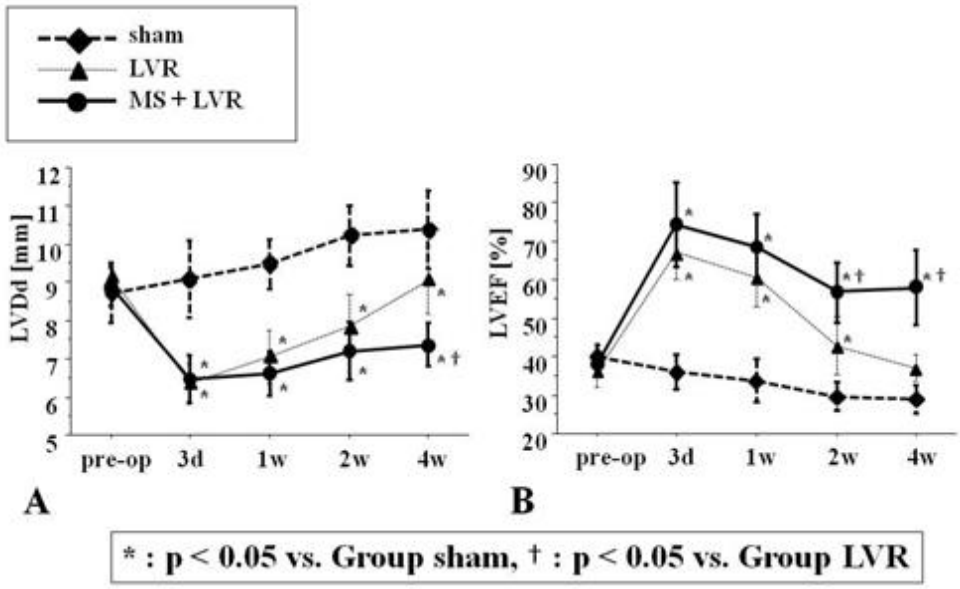
Rat myocardial infarction model was established 2 weeks after left anterior descending artery ligation. They were divided into 3 groups: sham operation (n=15; Group-Sham), LVR by plicating the infracted area (n=15; Group-LVR) and MS implantation with LVR (n=15; Group-LVR+MS).

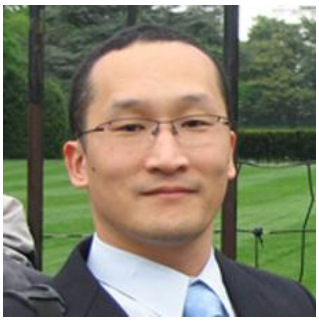
[Results]

Serial echocardiographic study revealed significant LV re-dilatation and decrease of ejection fraction 4 weeks after LVR in Group-LVR. MS implantation combined with LVR prevented those later deteriorations of LV function in Group-LVR+MS. Four weeks after the operation, a hemodynamic assessment using a pressure-volume loop showed significantly preserved diastolic function in Group-LVR+MS; end-diastolic pressure (LVR vs. LVR+MS: 9.0 ± 6.6 vs. 2.0 ± 1.0 mmHg, $p < 0.05$), end-diastolic pressure-volume relationship (LVR vs. LVR+MS 42 ± 23 vs. 13 ± 6, $p < 0.05$). Histological examination revealed cellular hypertrophy and LV fibrosis were significantly less and vascular density was significantly higher in Group-LVR+MS than in the other 2 groups. RT-PCR demonstrated significantly suppressed expression of transforming growth factor-beta, Smad2, and reversion-inducing cysteine-rich protein with Kazal morifs in Group-LVR+MS.

[Conclusions]

MS implantation decreased cardiac fibrosis by suppressing the pro-fibrotic gene expression, and attenuated the impairment of diastolic function and the late remodeling after LVR. It is suggesting that MS implantation may improve long-term outcome of LVR for ischemic heart disease.





Shunsuke Saito, MD, PhD
Department of Cardiovascular Surgery,
Osaka University Graduate School of Medicine,
Suita, Osaka, Japan

Promising Therapeutic Effects of Cell-sheet Technique with Pedicle Omentum Flap Through Angiogenesis-growth Related Self-renewal Mechanism for Ischemic Cardiomyopathy

Yasuhiro Shudo

Introduction: In the clinical study, we have proved that transplantation of skeletal myoblast (SMB) sheet onto the heart is effective and safe for ischemic cardiomyopathy (ICM), whereas this method still has limitations related with poor vascular network and cell-survival. We here hypothesized that wrapping of cell-sheet by the pedicle omentum, which is a vascular-rich organ delivering several kinds of stem cells and releasing a variety of angiogenic factors, might support survival of the transplanted cells and enhance the therapeutic effects of cell-sheet technique on ICM.

Methods: ICM model was generated by inducing anterior myocardial infarction for 4 weeks in mini-pig, while scaffold-free cell-sheet was generated from autologous SMB *in vitro*. The cell-sheets were then transplanted on the infarct area with or without pedicle omentum wrapping, while the mini-pigs that underwent either omentum flap only or sham operation were used as the control (n=6 each).

Results: The quantity of the iron-oxide labeled transplanted cells was significantly greater with omentum wrapping than those without omentum at 8 weeks after the treatment *(MRI)*. Transplantation of the cell-sheet wrapped with omentum significantly improved the regional myocardial deformation *(speckle tracking echocardiography)*, in association with the increased blood perfusion *(myocardial contrast echocardiography)* and vascular density; accelerate the growth of therapeutic vessels *(X-ray micro-CT and electron microscopy)* into the host ischemic myocardium; remodel the thin scar with thick well-vascularized cardiac tissues partially by reducing the infarct area. Consequently, the magnitude of global functional recovery was greater following transplantation of cell-sheet wrapped with omentum compared to that cell-sheet only.

Conclusions: The therapeutic effects of the myoblast cell-sheet wrapped with pedicle omentum flap enhanced angiogenesis-related self-renewal mechanism of cell-sheet technique, indicating a promise as a treatment for ICM.

Induced Pluripotent Stem Cell-Derived Cardiomyocytes Form Electrical Coupling with Native Myocardium Contributing to Functional Recovery in Rat Chronic Infarction Model

Takahiro Higuchi

Cardiovascular Surgery, Osaka University Graduate School of Medicine, Osaka, Japan

Introduction: Optimal delivery method of induced pluripotent stem cell (iPSc) into the heart has not been established; however, scaffold-free cell-sheet method has been shown to effectively deliver abundant functional cells, whereas functional integrations of implanted cell-sheet into native myocardium are not fully addressed. We here hypothesized that iPSc-derived cardiomyocytes (CM) implanted with cell-sheet method might form electrical coupling with the native myocardium contributing to functional recovery in chronic myocardial infarction.
Methods: iPSc-CM of mouse origin was generated by culturing embryoid body. The chronic infarction model was generated by occluding the left coronary artery for 2 weeks in immunodeficient rats, which then underwent either implantation of cell-sheet generated by iPSc-CM, neonatal CM, fibroblast or skeletal myoblast over the infarct area, or sham operation with the effects assessed until 14 days post-treatment.
Results: Daily electrical mapping of the heart surface using 64-channel multi-electrode probe uncovered that the iPSs-CM and the neonatal-CM treatment induced multiple ectopic excitations, which were not connected to the native myocardial electrical current, over the cell-sheet implanted area until 2 days. Interestingly, from 3 days onwards, ectopic excitations disappeared and magnitude of myocardial electrical current increased following the iPSc-CM or the neonatal-CM treatment, suggesting that electrical stimuli of the native heart were properly transferred into the cell-sheet. Consistently, left ventricular ejection fraction (LVEF) and activation recovery interval (ARI) of the iPSc (54%, 68 ms) and the neonatal CM treatment (54%, 71 ms) were significantly improved at 3 days compared to LVEF and ARI following the fibroblast (49%, 92 ms), the myoblast (49%, 89 ms) or the sham (48%, 96 ms). Electron microscopy and immunostaining identified the ultrastructure of the implanted iPSc-CM.
Conclusions: The iPSc-CM sheets may electrically integrate into the native myocardium in 3 days after implantation and contribute to functional recovery in rat myocardial infarction model.

Takahiro Higuchi, MD, cardiovascular Surgery, Osaka University Graduate School of Medicine

Myoblast cell sheet therapy avoids inflammation and arrhythmias in ischemic heart failure

Pätilä Tommi1),2), Miyagawa Shigeru1), Imanishi Yukiko1), Fukushima Satsuki1), Siltanen Antti3), Kankuri Esko3), Harjula Ari2),Sawa Yoshiki1)

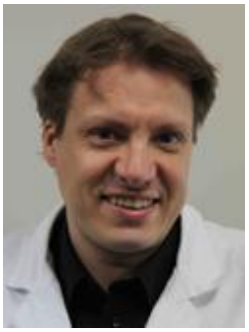
1) Osaka University Hospital, Japan
2) University of Helsinki Meilahti Hospital, Finland

Study objectives
Myoblast injections in failing myocardium might cause arrhythmia. We hypothesized that inflammatory
reactions and cell-clusters by direct intramyocardial injection of myoblasts generate electrical re-entry and this could be avoided by transplanting myoblast sheet on the surface of the heart.

Methods and material
Infarcted rat hearts received direct injection of myoblasts, topical application of scaffold-free myoblastsheet or sham operation.

Results
Left ventricular dilatation was attenuated in cell injection group and cell sheet group with EF improvement in cell-sheet group, whereas sham group deteriorated. The number of ventricular premature
contractions (VPCs) was significantly higher in the injection group at 1 day and 14 days after treatment
when compared to the control animals ($p<0.05$ and $p<0.01$, respectively) and furthermore, the VPCs were significantly higher in the injection group at day 7 and day 14 ($p<0.05$ and , $p<0.01$, respectively)
when compared to the sheet group. There were no spontaneous ventricular tachycardia (VT) recorded in any of the animals. Epicardial electropotential mapping under Isoproterenol stress showed macro-re-entry at infarct border area leading to VT in injection group animals, but not in the other groups ($p=0.045$). Expression of IL-10, IL-1s and IL-12 genes were higher in the injection group compared to the sheet group with high accumulation of inflammatory cells in the injection areas.

Conclusion
Inflammatory reactions at the myoblast injection areas cause inducible re-entry circuits. Transplantation of scaffold-free cell constructs on the surface of the heart might be optimal delivery method of skeletal myoblasts to treat heart failure.



Tommi Pätilä, MD PhD, Department of Cardiothoracic Surgery,
Helsinki University Central Hospital

MYOCARDIAL CELL RETENTION AND BIODISTRIBUTION AFTER CARDIAC CELL TRANSPLANTATION

I. Kutschka, A. Martens, S.V. Rojas, H. Baraki, R. Zweigerdt, U. Martin, A. Haverich

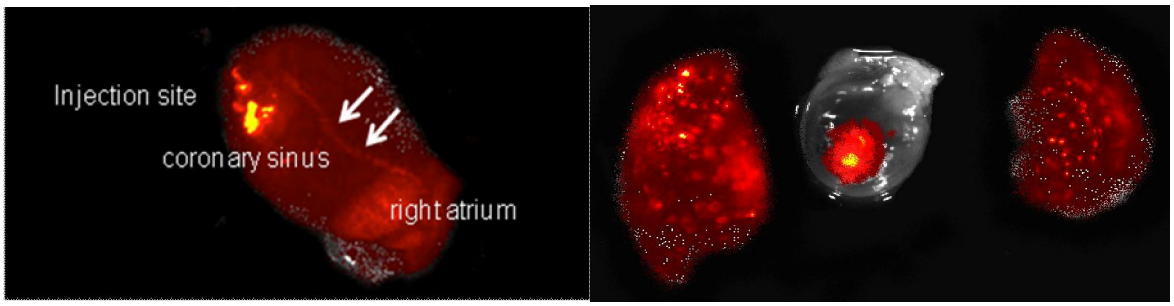
Background: Successful stem cell delivery and subsequent biodistribution are underestimated but critical factors for the efficacy of cardiac stem cell therapy. Myocardial cell retention is known to be low after direct injection. We compared quantitative techniques to evaluate stem cell loss and biodistribution after intramyocardial application using fluorescent microspheres. We transferred these techniques to a cellular system using fluorescent and bioluminescent iPS cells in a mouse model of acute myocardial infarction. Fibrinogen as a viscose carrier substance was evaluated to augment intramyocardial particle and cell retention.

Methods: Following LAD ligation 5 x 10^5 fluorescent microspheres (d=10µm) were transplanted into 16 mice hearts in two groups (A=particle suspension in PBS, B= particle suspension in fibrinogen). 10min after injection the hearts were explanted and fluorescent imaging was performed using a Xenogen IVIS Lumina-System. Hearts were subsequently homogenized, filtered and aliquots were again analysed using fluorometry, fluorescence imaging and a counting-chamber.
Murine induced pluripotent stem (iPS) cells were transfected for luciferase reporter gene expression and transplanted into infarcted myocardium after LAD ligation in mice (1 x 10^6 cells/15µl). Cells were delivered in aqueous media (PBS; group A; n=15) or fibrinogen (group B; n=15). First, grafts were detected *in vivo* 10 minutes after transplantation using bioluminescence imaging (BLI , IVIS Lumina, Xenogen). To further quantify detailed early cell retention and organ biodistribution hearts and lungs were harvested and analyzed 15 minutes after injection.

Results: Direct intramyocardial injection of fluorescent microspheres (5 x 10^5) in PBS showed a 90% loss of particles in terms of cardiac particle retention (0.33 x 10^5) indicating immediate loss during the cell grafting procedure. This insufficient cell delivery is caused by a significant number of cells remaining in the tip of the syringe and a relevant particle backflow through the injection channel. Furthermore, we observed a significant redistribution of microspheres mainly through venous drainage into the lungs. Microspheres were distributed by one third in the heart and by two thirds in both lungs. Particle retention in the heart could be significantly improved using fibrinogen (0.33 x 10^5 vs. 1.08 x 10^5; $p<0,05$) as particle carrier. Using bioluminescent murine iPS cells for transplantation these findings could be confirmed. BLI showed a marked venous cell drainage into the right atrium. Consequently, cardiac retention of injected cells was low (24 ± 2%). Cells accumulated in the right lung (52 ± 3%) and left lung (24 ± 4%). Fibrinogen significantly increased cardiac iPS cell retention when compared to PBS (71 ± 4%, $p < 0,01$). Distribution to the right lung (17 ± 3%) and the left lung (12 ± 2%) were also significantly lower in the fibrinogen treated group ($p < 0.01$).

Conclusion: Macroscopic fluorescent and bioluminescent imaging proved to be a fast and reliable tool to provide additional morphological information after intramyocardial microsphere and cell application. We unveiled massive extracardiac loss of fluorescent microspheres and iPS cells after intramyocardial injection in

aqueous media. Venous drainage leads to a pulmonary accumulation of transplanted cells. Fibrinogen as a viscous carrier medium improves cell retention within the cardiac target area.



A B

Fig.1: Redistribution of fluorescent microspheres directly after injection into infarcted rat myocardium. Microspheres are washed out via the venous system (A) resulting in pulmonary embolization (B).

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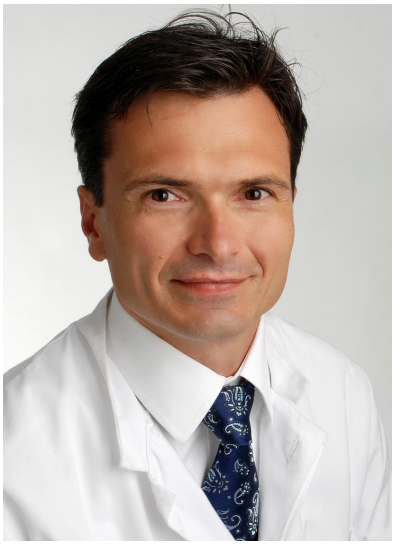
Hannover Medical School
Hannover, Germany



Bone marrow stem cell-derived cell sheet transplantation for myocardial infarction in rats -A comparison with skeletal myoblast sheet transplantation

Yukiko Imanishi

Background:
For treatment of heart failure, the cell delivery using cell sheets showed better restoration of damaged myocardium compared with needle injection. Cell sheet transplantation is a way which maximizes paracrine effects, but optimal cell source to generate cell sheet has not been addressed. Bone marrow-derived mesenchymal stem cell (MSC) and skeletal myoblast (SMB) are versatile cell source in clinical trial, which have different cellular properties. We hypothesized that the difference of the cellular property between MSC and SMB results in different impacts after cell-sheet transplantation on sub-acute infarct heart. Thus, we compare the cellular property and effects of MSC and SMB on the infarct heart to explore optimal cell transplantation therapy.

Methods and results:
We created cell-sheet from Lewis rats derived MSC and SMB. MSC-sheet was thin compared with the SMB-sheet (43 +/- 11 vs. 123 +/- 23 μm). Gene expression array showed lower expression of extra-cellular matrix related genes as collagens in MSC. Either the two layers of MSC- or SMB- sheet were implanted on the left ventricular anterior wall (n=12 each) or only chest-open (control, n=11) at 2 weeks following permanent LAD occlusion in Lewis rats. Four weeks after treatment, echocardiography showed EF was significantly higher in the both cell-transplanted groups compared with the control. Histological analysis showed that infarct size was smaller in the SMB group and capillary density was higher in the MSC group. Fibrosis at infarct-border zone was significantly lower in the both cell-transplanted groups. Arrhythmogenicity monitored by electrocardiogram showed the event frequency of abnormal premature ventricular contraction was not different in the groups.

Conclusion:
MSC-sheet transplantation showed functional recovery after myocardial infarction, which may be mediated by the different mechanism such as enhanced blood restoration from SMB-transplantation.

Figure 1. Cardiac performance of MSC- and SMB-treated myocardial infarction rats.

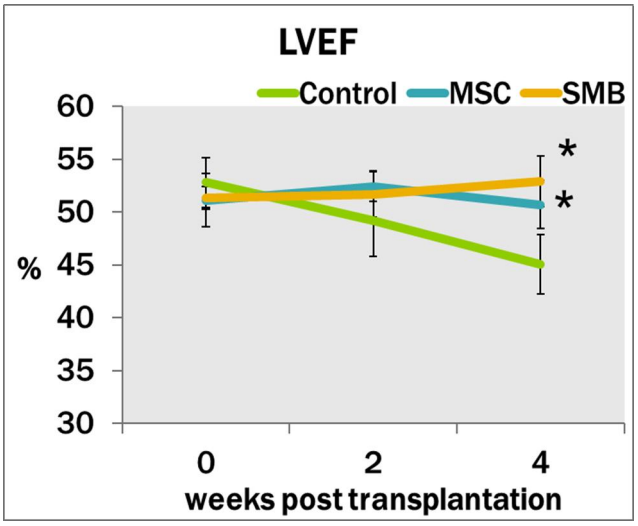
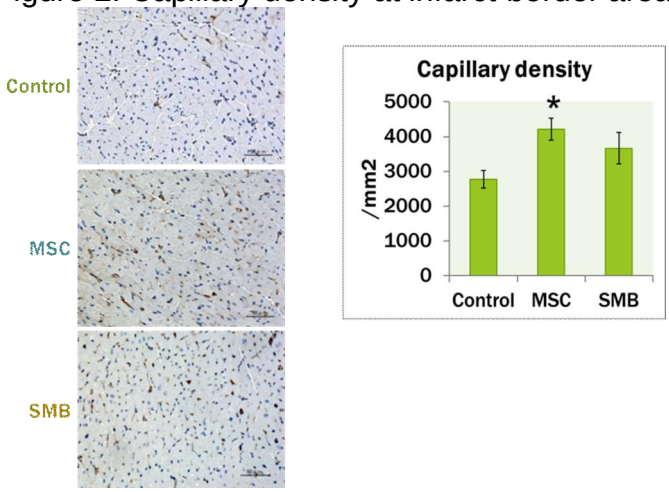
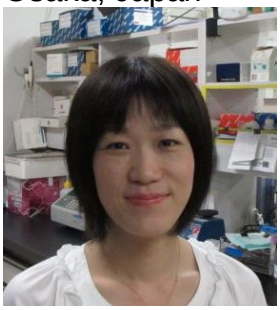


Figure 2. Capillary density at infarct-border area at 4 weeks after cell transplantation



Yukiko Imanishi, PhD.
Department of Surgery, Osaka University Graduate School of Medicine
Osaka, Japan



INTRAMYOCARDIAL TRANSPLANTATION OF BIOARTIFICIAL CARDIAC TISSUE SPLINTS FOR RESTORATION OF INFARCTED MYOCARDIUM IN RATS

H. Baraki, I. Kutschka, I. Gruh, A. Haverich

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.

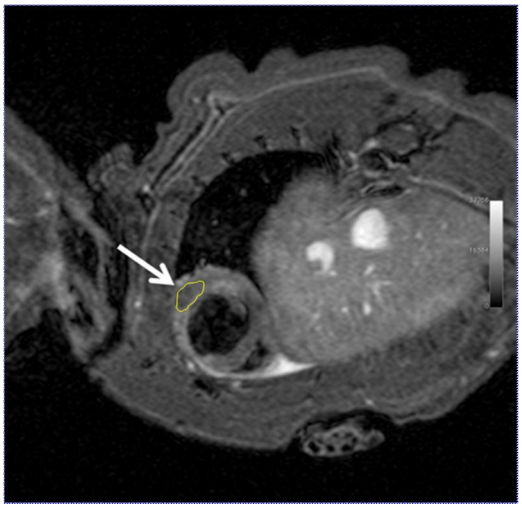
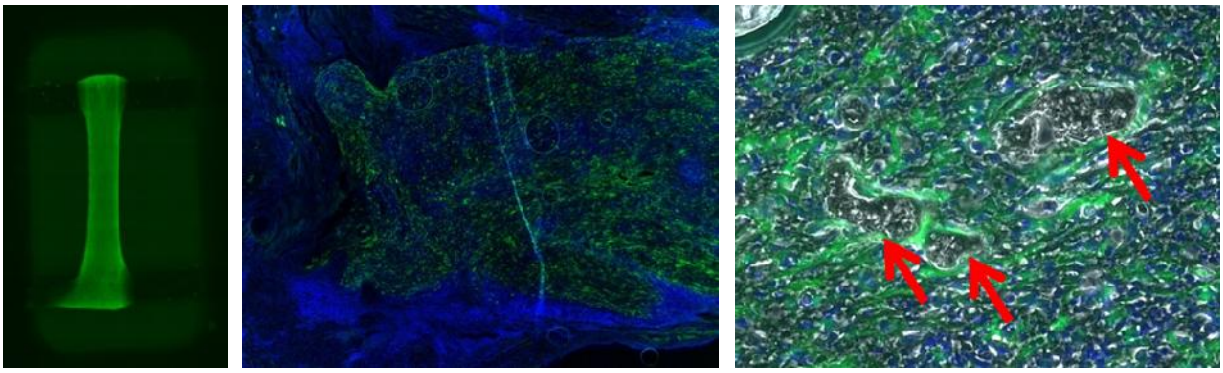


Fig.1: Short axis MRI image of an infarcted rat heart four weeks after intramyocardial BCT implantation (arrow).



A B C

Fig.2: (A) BCT based on neonatal cardiomyocytes from GFP rats before intramyocardial implantation. (B) DAPI staining of BCT after intramyocardial implantation. (C) Erythrocytes inside de novo vessels of the BCT 4 weeks after intramyocardial transplantation. Blue: DAPI staining of cell nuclei, green: GFP

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Preliminary studies with Vineberg procedure in rat myocardium

Regenerative medicine provides modern treatment methods, such as cell sheet therapy, for cardiac patients. Yet current methods are not totally optimized. One major difficulty in cell sheet therapy is to keep the cells alive. One way to increase the survival would be to provide the sheet with external blood circulation. We developed a model to study angiogenesis in rat heart. A so called extra coronary artery was provided to the heart by Vineberg operation. The left internal thoracic artery became patent in 4 weeks post surgery. Histology showed induced angiogenesis at the area of anastomosis. Added external circulation could provide a novel method to improve the outcome of cell sheet therapy.

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Everolimus enhances allogeneic myoblast sheet therapy in rat acute myocardial infarction

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Transplantation of allogenic, instead of autologous, myoblasts allows use of therapeutically defined and optimized cell therapy for cardiac injury. Because no lag time for cell expansion is needed, an off-the-shelf allogenic cell therapy product would enable flexible timing for the administration of treatment. Immunorejection, however, is a major hurdle for allogenic myoblast therapy. We evaluated the ability of the immunosuppressive mTOR inhibitor everolimus to prevent rejection of allogenic myoblast sheets in a rat model of acute myocardial infarction (AMI). To evaluate sheet survival *in vivo*, L6 myoblast sheets expressing firefly luciferase were transplanted subcutaneously to Wistar rats receiving 1 or 3 mg/kg/day everolimus. Control rats received placebo. We followed sheet survival for 4 weeks with *in vivo* bioluminescence imaging. Bioluminescence imaging revealed decreased sheet survival by 11 days and by 14 days sheets were not detected. Everolimus prolonged sheet survival and at 4 weeks, the 3mg/kg/day group showed no decline in sheets survival. We then transplanted L6 sheets to rat hearts after inducing AMI by left anterior descending coronary artery ligation and evaluated the ability of everolimus to enhance cardiac function. In the AMI hearts, animals receiving everolimus had enhanced cardiac function as compared animals receiving sheet transplantation and placebo. In conclusion, in an allogenic setting mTOR inhibition can prolong sheet survival and enhance efficacy of myoblast sheet transplantation therapy.

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