JSPS Core-to-Core Program

Abstract Brochure

2013 Core-to-Core Program International Symposium for Young Researchers

11th December 2013
15:00-18:00
Seminar room, Clinical Research Institute 3F
Osaka University, Graduate School of Medicine, Japan

Program

Title: Five years results and future scope of our Core-to-Core program

Moderator: Masamichi Ono： Hannover Medical School

Opening Remarks：15:00 - 15:05

Yoshiki Sawa: Osaka University

Greeting from German Coordinator: 15:05-15:10

Axel Haverich(video) Hannover Medical School

Greeting from Finnish Coordinator: 15:10-15:15

Ari Harjula(video) University of Helsinki

Greeting from JSPS: 15:15-15:20

Yukiko Abe: Japan Society for the Promotion of Science

Part 1: Summary of Core to Core Program 2009 - 2013 : 15:20 – 15:50

Myocardial regeneration and Cell Sheet therapy:

Shigeru Miyagawa: 15:20 - 15:40 Osaka University

Consortium development between Helsinki, Hannover, and Osaka

Masamichi Ono: 15:40 - 16:00 Hannover Medical School

Coffee break: 16:00 – 16:20

Part 2: Special Session for Tissue Engineered Valve：16:20‐17:55

Moderator: Takayoshi Ueno： Osaka University
Masamichi Ono： Hannover Medical School

From bench to bedside: Construction of autologous heart valves based on acellular allograft matrices

Karolina Theodoridis: 16:20-16:40 Hannover Medical School

Tissue engineered valve first clinical results in pulmonary position

Dietmar Boethig: 16:40-17:00 Hannover Medical School

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

Samir Sarikouch: 17:00-17:20 Hannover Medical School

Decellularization and logistics of heart valves

Michael Harder: 17:20-17:40 Hannover Medical School

Tissue engineering valve project in Osaka University

Hideto Ozawa: 17:40-17:55 Osaka University

Future prospective and closing remarks: 17:55 -18:00

Yoshiki Sawa: Osaka University

Yoshiki Sawa, MD, PhD.

Professor and Chairman
Department of Cardiovascular Surgery
Osaka University Graduate School of Medicine
Osaka, Japan

Greeting from the Japanese Coordinator

Dear Colleagues,

It is my great pleasure to hold this educational international symposium for young researcher of this year in Osaka University. This program is supported by JSPS's Core-to-Core Program, Rebirth program of Deutsche Forschungsgemeinschaft (DFG), and Bilateral program of Academy of Finland (AF).

This Core-to-Core Program between Finland, Germany, and Japan, has been started since 2009. Following to the previous meetings in Vienna, Geneva, Tampere, Nagoya, Yokohama, Hannover, and Helsinki, this international symposium is the last event of the 5-years scheme of Core to Core program. I would like to exchange most recent results of our research and also clinical translational works on myocardial regeneration and cardiovascular tissue engineering, especially on the clinical tissue engineered pulmonary valve project at this time, and make fruitful discussion for the further development of new technologies and methods that open the doors for completely new possibilities of patient-specific treatment.

Cardiovascular and transplant surgery has changed dramatically in the past few years. The development of new technologies and methods has enabled huge advancements that open the doors for completely new possibilities of patient-specific treatment. Cardiovascular tissue engineering is one of the promising fields to be developed, and is expected to produce ideal materials for cardiovascular and transplant surgery. Our core institutions have developed unique technologies in this field, and some of them have been clinically applied. In our international consortium, we will unite and integrate our technologies and produce ideal materials for supplement cardiovascular defects or support cardiac function.

I hope this international szmposium makes great progresses for developing our international research consortium for myocardial regeneration and cardiac tissue engineering.

Sincerely,

Yoshiki Sawa

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

Good afternoon, ladies and gentlemen,

I have the privilege to have developed this international research consortium for cardiovascular tissue engineering and myocardial regeneration between Helsinki, Osaka, and Hannover in the scheme of Core to Core Program.

Unfortunately, I am unable to fly to Japan this time of the year, but I am proud of the 5 years successful results. We have made many meetings in various locations, on the international ground, in various countries including Germany. In my view, the core to core program has been extremely successful. We have not only exchanged the idea and the concept of myocardial regeneration and cardiovascular tissue engineering, but now we are face to reach the stage we can also exchange the clinically applicable products of regenerative medicine between the countries and between the centers, and it is, I think, a single success. Congratulation and thanks to all of you.

However, in this context, we are not finished. If we want to go into cellular replacement strategy by means of tissue engineering using differentiated stem cells, it would be difficult before clinical treatment. This will be our task in the coming years. Congratulations on this success to Japanese society of regenerative medicine and the president of this society, Prof. Sawa. I am very delighted, that so much of things we can perform in this consortium.

In the recent Herrenhausen symposium on stem cells and regenerative medicine in Hannover, I have met Prof. Masayo Takahashi who applies the first iPS cell product to human, and she is very proud of Prof. Sawa is the clinician who explore the clinical application of stem cell products in Japan with the aid of Japanese government. I am proud that Prof. Sawa is one of the key persons of our consortium.

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

Best regards,

Axel Haverich

Ari Harjula, MD, PhD.

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

Greeting from the Finnish Coordinator

Dear friends, our members of our consortium,

Warm greeting from Helsinki! I have the privilege to initiate this international research 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 Finland, Japan, and Germany, we will for sure have extended results and innovations to report in this final Osaka meeting. I am very delighted, that so many of your colleagues, seven doctors and scientists from Osaka have stayed in Helsinki not only for research works but also for clinical works, and that they have been able to participate in our communities. We have performed also post doctoral exchange, our colleagues in Helsinki stayed in Osaka to develop their carriers.

The recent progress in cardiac tissue engineering and cell sheet technology will encourage us to further development of our research programs. I hope our granting could be expanded in the coming years and that we can promote further exchange of our young students and post doc fellows makes real co-operation and understanding of our cultural and research as well as clinical practices even more realistic. Adding the Hannover team, our consortium has developed so innovative and proliferative.

I am sure that we can expand this international research consortium in the future, and we can meet together and exchange our research results, exchange the colleagues, and also exchange the research and clinical products as we have done in the last five years.

Best regards,

Ari Harjula

Yukiko Abe

Head, Research Cooperation Division
International Program Department
Japan Society for the Promotion of Science

Message from JSPS

We send our hearty congratulations to all of you on this occasion of the 2013 Core-to-Core Program International Symposium for Young Researchers, titled “Five Years Results and Future Scope of our Core-to-Core program.” To Prof. Yoshiki SAWA of Osaka University and each of you who have worked so hard to implement this superb event, we very much appreciate your dedication and efforts.

The Japan Society for the Promotion of Science is Japan’s leading funding agency supporting scientific research based on the researchers’ own free ideas across a full spectrum of fields. JSPS provides a wide range of programs geared to advance next-generation scientific advancement. Among them, programs to promote international scientific exchange are given high priority. The JSPS Core-to-Core Program, under which this seminar is being held, aims to create world-class research hubs while fostering highly talented young researchers. It does this by supporting networking aimed at promoting multilateral collaboration in cutting-edge fields of science among the most advanced research institutions.

To invigorate and elevate research within a global environment, there is a vital need for young researchers to participate in multi-national projects of a caliber capable of creating new knowledge and making milestone research advances. They need to begin accruing this experience at an early stage of their careers.

JSPS has designed this program in such a way as to give doctoral students and young researchers a valuable developmental experience as they set out on their path to becoming the next generation of frontline researchers who will play active international roles and advance joint research of importance to society.

Coordinated by Prof. SAWA, your project “Construction of Cardiac Tissue Using Integrated Gene, Cell, and Tissue Engineering Technology and Its Application for the Treatment of Cardiac Failure”, has marked outstanding research achievements both in terms of its scientific advancement, societal contribution, and high-quality education. This will be the last year of the project under the Core-to-Core Program. I understand that in this symposium the participants will present their results. I look forward to receiving a report on your presentations, which I am sure will compile and amplify the project’s very fruitful outcomes over the duration of its implementation to date.

As the next stage, we at JSPS look forward to you building upon your achievements and continuing to advance your joint research activities in ways that will create powerful regenerative medicine platforms.

We extend you our best wishes for utmost success in this symposium as well as in your studies and individual research endeavors.

Shigeru Miyagawa, MD, PhD.

Principal Investigator
Dept. of Cardiovascular Surgery
Osaka University Graduate School of Medicine
Osaka, Japan

Myocardial regeneration and Cell Sheet therapy:

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.

Masamichi Ono, MD, PhD.

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

Consortium development between Helsinki, Hannover, and Osaka

It is my great pleasure to welcome you to the 8th meeting of Core-to-Core Program at Osaka University Graduate School of Medicine, the Japanese Core Institute. Since 2009, our international research consortium has made many progresses by the scheme of Core-to- Core Program. At this meeting in Osaka, we summarized our activities of the 5 years successful results, and discuss the idea and the concept of our future figures of our consortium.

During the last 5 years, we have made many meetings in many countries, have exchanged about 20 young researchers between the institutes, and have performed international collaborative research works. Through these activities, we have expanded and strengthened the research networks that will build strategic interdisciplinary research hubs in centered Japan, established sustainable research partnerships between research institutions in Japan and those in Germany and Finland, and contributed to fostering young researchers who will advance the next generation of science. Totally more than 100 million yen has been granted, and the more active research works has been expected including summer seminar and international symposiums for young researchers.

In my presentation, I would like to show you our 5 years results of our activities and let you know the development and expansion of our international research consortium by core to core program. I hope further development of our research consortium in the coming years, which bring us the real international research and clinical hubs in the field of myocardial regeneration and cardiovascular tissue engineering.

Karolina Theodoridis, MD.

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

From bench to bedside: Construction of autologous heart valves based on acellular allograft matrices

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.

Dietmar Boethig, MD, PhD.

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

Tissue engineered valve first clinical results in pulmonary position

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 risk 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 decellularization 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.

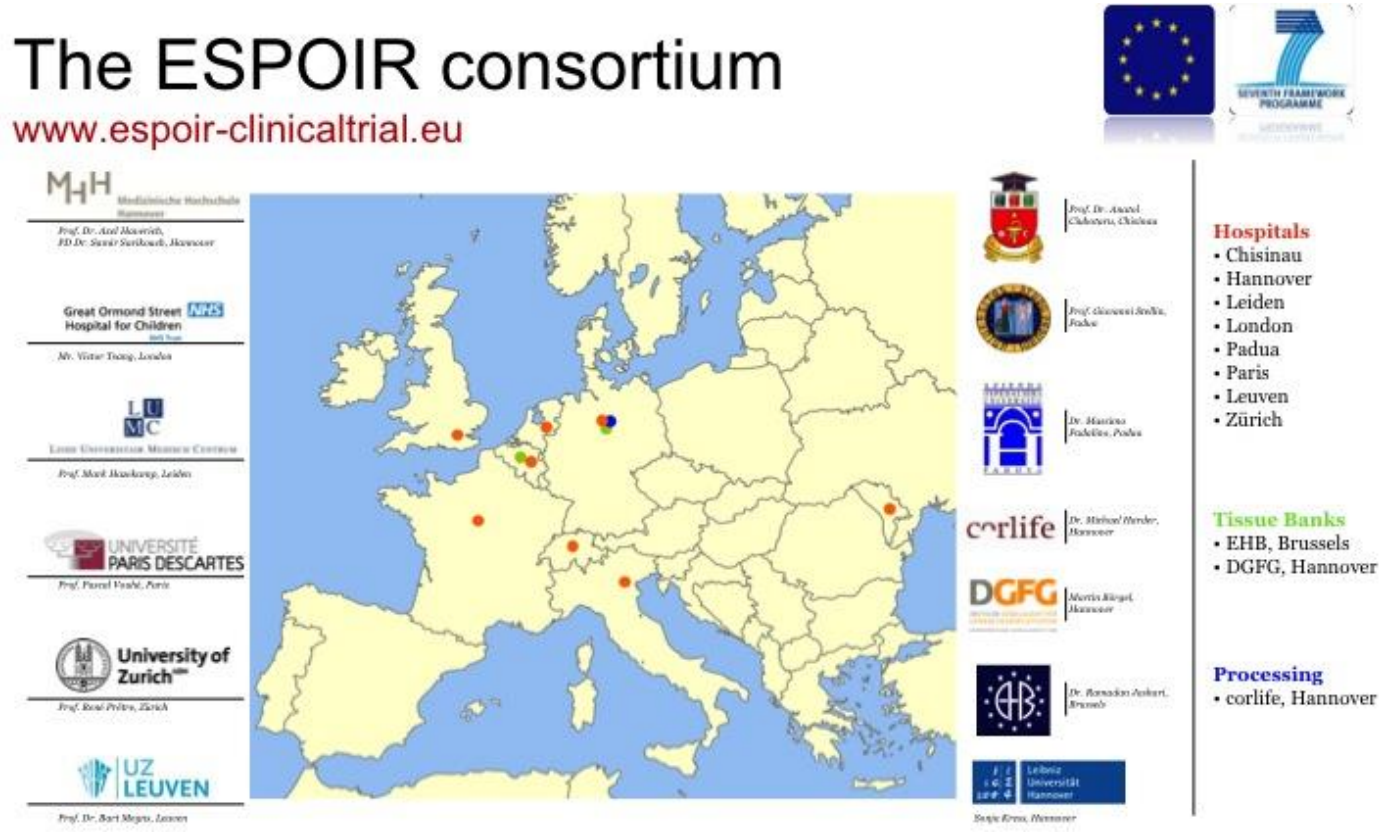
The main objective of the proposed ESPOIR project is translation of the innovative regenerative therapy presented by the DHV regenerative heart valve into widespread clinical use. To achieve this goal, the ESPOIR consortium will:

- 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.



Michael Harder, PhD.

Corlife oHG
Managing Partner
Hannover, Germany

Decellularization and logistics of heart valves

Corlife is a tissue establishment and medical device company based in Hanover, Germany. Founded in 2006, corlife is specialized in cutting-edge stented and stentless devices based on tissue-engineered matrix composites which, once implanted, are integrated and become indistinguishable from the patient's own tissue. All products are produced in our company's clean rooms in accordance with a detailed quality management system which fulfills all current European standards.

Human heart valves are capable of regeneration and, as such, are to a great extent resistant to abrasion and infection. Transplanted valves can provide reliable substitutes and have been successfully used to save the lives of many patients. However, these valves often induce a gradual immunological reaction which ultimately prevents the complete integration and regeneration of the heart valve. Decellularized heart valves present an alternative to conventional valve transplants as these are progressively integrated in the patient's own tissue and thus harbor the potential for regeneration.

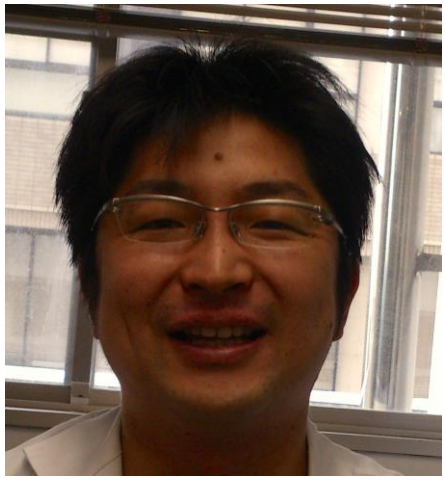
All original cells are removed from the explanted valve in a complex physical/chemical process, leaving a stable heart valve structure consisting of collagens and elastic fibres. The entire process is closely controlled to ensure the complete safety of the transplants. Decellularized heart valves have been successfully and safely used in clinical practice for over ten years. The decellularized human Pulmonray valve, Espoir PV, is an approved product in Germany, Italy and Switzerland. Application other counties in Europe are ongoing.

The decellularized human Pulmonray valve, Espoir PV, is shipped in 0,9% NaCl at 0°-10°C. The shelf life is currently 50 days.

Human tissue is subject to a prohibition on trade and commercialization. As a result, it is not possible to order decellularized human tissue products directly from corlife. Enquiries must be directed to the relevant tissue bank supplying the hospital in question. The tissue bank can then allocate an individual heart valve which can be forwarded to corlife for decelluarization. corlife has no influence on the allocation procedure. corlife receives a fee for the decelluralization commensurate with the costs and business risks involved in product development.

Hideto Ozawa, MD.

Dept. of Cardiovascular Surgery
Osaka University Graduate School of Medicin
Osaka, Japan



Tissue engineering valve project in Osaka University

We prepare the clinical using of the tissue engineering heart valve for congenital heart disease in Osaka University. For starting of this project, we already had an approval of this project and we are undergoing preclinical experiments with animals. We present our data of the preclinical study in Osaka University.
Method and Resuts: First, we underwent decellulalization of right ventricle tract of the porcine. We used the protocol same as Hannover medical school was reported. 10 grafts were decellulalized and confirmed the decellularlization by histology. We implanted theses grafts for porcine models of 5 cases, and 3 cases could survive after the operation(one for bleeding , one for infection). By echocardiographic and MRI examination, implanted valve function maintain 2 month after the surgery. Extra cellular matrix of the implanted valves were maintained and detected by elastin, smooth muscle actin, collagen I and IV, by immunohistocehmical staining. Also, endothelial cells were detected in implanted valve at anastomosis site and the surface of the valve leaflet by anti CD31 antibody. Next, we extracted right ventricle tract from the recipient's heart at the time of heart transplant, in satellite condition. Total 7 graft were extracted (LVAD 6, BiVAD 1) until now. Pulmonary valve leaflet looked normal formation in LVAD cases(DCM 4, dHCM 3), however, in BiVAD case (ARVC), the commissure of 3 leaflets were fusioned and could not used for graft. The other 6 cases, graft were decellulalized as same protocol in satellite condition and stored at 4℃ temperature. Decellularlization were confirmed by histology and extra cellular matrix of the implanted valves were maintained and detected by elastin, smooth muscle actin, collagen I and IV, by immunohistocehmical staining. One of the valve (DCM with DuraHeart , annulus size 26mm) was implanted at anatomical position of the porcine model without administration of the immunosuppressant drugs. 2month after the surgery, pulmonary valve function was maintained and echocardiography and MRI detected regurgitation or stenoses were not detected.
Conclusions: Preclinical testing of the tissue engineering valve project was satisfactory. Possibility of the xenograft implanatation may be required further more experiments.