New Energy and Industrial Technology Development Organization (NEDO) has selected: Reacceleration of Yamaton H project

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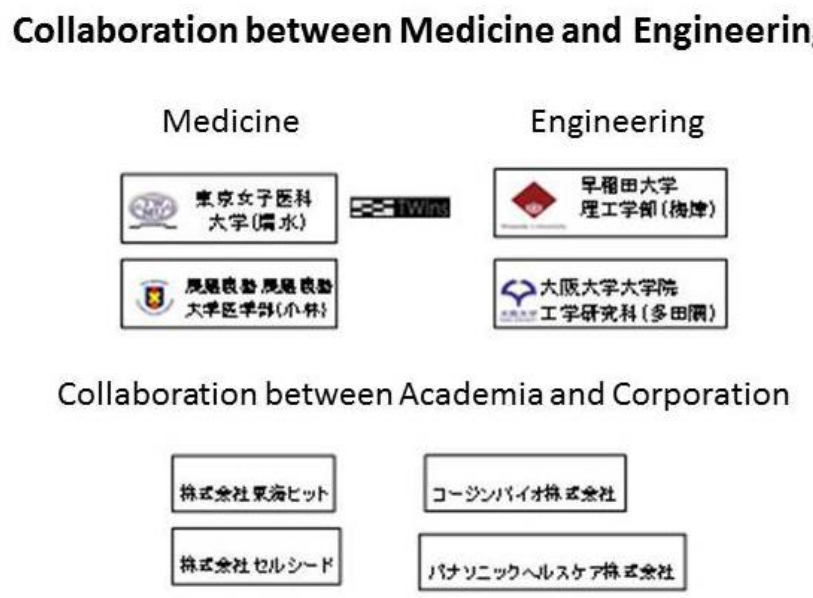
NEDO selected on November 5, 2014 the co-research with Dr. Shimizu of Tokyo Womenôs Medical University entitled ñThe development of functional live tissue production technology through 3-D moldingò which focuses on the research and development of functional 3-D organ fabricating technology. Below is the outline of the project as well as the historical path of mine in regard to the research. I start co-research for this NEDO project for a period of 5 years in collaboration with Dr. Shimizu and Dr. Sekine of Tokyo Womenôs Medical University by merging the technology of developing vascular bed with large-scale inflow blood vessel.

Outline of the project

The main objective of this research is to further develop a production technology for 3-D tissue construction based on cell sheet engineering originated by Dr. Shimizu of Tokyo Womenôs Medical University as a project leader which is Japan original, first time ever in the world and to firmly establish a production technology and know-how for 3-D organs with blood vessels for clinical application. We create partially flat-surface heart with vascular plexus and tubular heart with pumping function by utilizing a newly developed in-vivo vascular bed in which human myocardium sheets are gradually multilayered and cultured circumfusion in the bioreactor resembling living organ environment. We establish a functionality evaluation system for the fabricated organs electrically, physically (pulse and pressure), and metabolically as well as we verify the effectiveness through the transplantation to disease models. Furthermore, we aim at widening the application of the organ fabrication system to other organs such as liver and kidney. And the final goal in the future is to create revolutionary regenerative medicine to subsidize for organ transplantation.

Framework of research: Medicine-Engineering and Academia-Corporation



Eiji Kobayashiôs role for the project

Kobayashiôs role is to develop vascular bed which acts as a scaffold for structuring blood vessel for clinical applications. I create partially flat-surface heart with vascular plexus and tubular heart with pumping function by utilizing a newly developed in-vivo vascular bed in which I select biological tissue and organs cultured circumfusion in the bioreactor where we can verify stable in-vitro perfusion. The former is supposed to be greater omentum or mesentery, the latter; small intestine. Furthermore, in-vivo vascular bed is fabricated for composing cell sheetsômultilayer through the decellularization in bioreactor and reseeding of vascular endothelial cell into vascular cavity. It is considered to seed human mesenchyme cell or fibroblast into the interstitial subdivision. In the attempt to fabricate functional partial heart or tubular one, my objective is to structure vascular bed suitable for matching the part for transplantation and synchronizing with cardiac beat. As for the flat-surface heart I judge the size suitable for clinical application from the beginning. The size of the tubular heart is judged through R&D by using small animals and will be finalized in the latter half of the R&D period taking clinical application into consideration.

Elaboration for structuring multilayered myocardium sheets based on cell sheet engineering in collaboration with the team of Tokyo Womenôs Medical University

So far, the team of Dr. Okano as a team leader has been pursuing for tissue regeneration based on the cell sheet engineering. The acquisition of cell sheet becomes possible through the process of lowering temperature in the temperature-corresponding-culture dishes. (CellSeed Inc.) The fabrication of high density cells through multilayer technology which totally excludes bioreabsorbable vascular scaffold. The cell sheet transplantations have already started in shape of single-layer to multi-layer with clinical applications for diseases of cornea, heart, esophagus, periodontal, cartilage and middle ear.

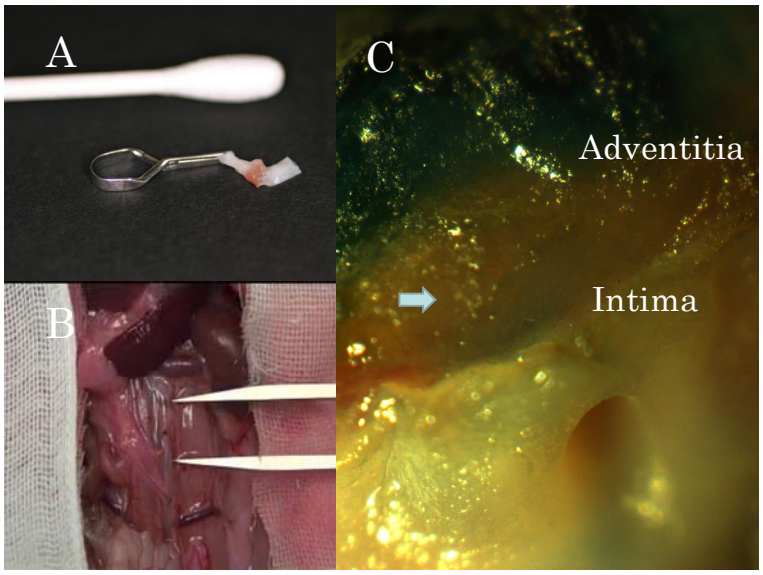
On the other hand, concerning the establishment of 3-D tissue and organ, it is proven that the limit of multilayer is three with 100um in thickness due to the insufficiency of oxygen and nutrition. I have been elaborating to prove the concept through the experiments of growing in-vivo myocardium sheet to be multiplied as myocardial tissue (Shimizu T, FASEB J 2006). Also created a rat model with beating vessel by binding myocardium sheet around the abdominal aorta (Sekine H, Circulation 2008). As this beating blood vessel shows more than 10mmHg in pressure, we have shown the possibility of clinical application by utilizing it in the venous system first time ever in the world. Currently for small animals within in-vivo subcutaneous tissue, 1mm thickness functional myocardium tissue is created by transplanting repeatedly (graded lamination method) up to 30 layers of vascular endothelium and myocardial culture sheet (3 layers) (Sekine H, Nature Comm 2013).

Hereafter, it is indispensable to invent a technology of 3-D organs applicable for clinical science to fabricate large-scale vascular bed with capillary plexus.

Trial for fabricating vascular bed with large-scale blood vessel by using pig model

There have been lots of challenges to overcome the difficulties in cell sheet engineering while it has been applied for clinical purposes. I have been in need of verifying with an animal model like pig whose body size is equal to that of human in order to fabricate heart applicable for human.

The proof of concept using small animals is scientifically important but is not applicable for the experiments with large animals. When I succeeded in fabricating a rat model with beating vessel in 2007, right after the success I tried to structure a beating vessel with one cardiac chamber and one auricle.

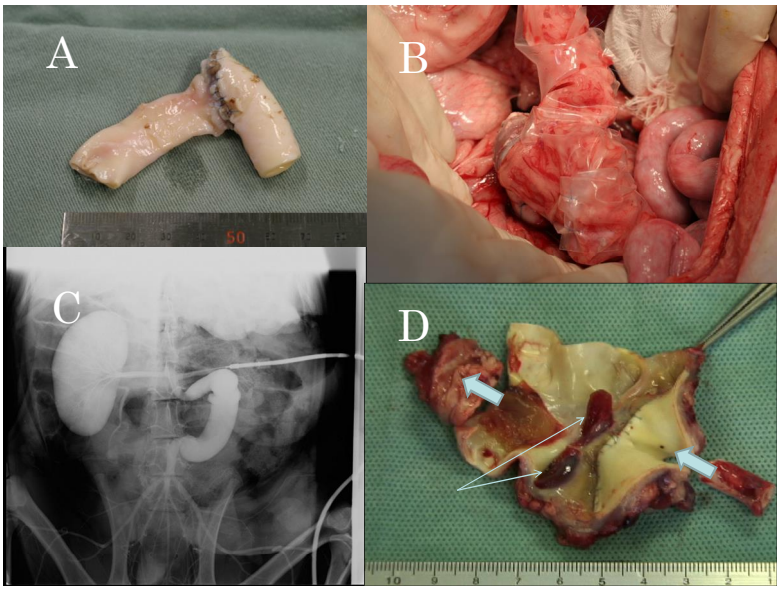


A: Rat blood vessel chamber with pulmonary artery valve; the decellularization of pulmonary artery was possible because it had low pressure valve separated and connected with artery.

B: Replacement of artery for blood vessel chamber; matured LEW (genetically-modified rat with LacZ gene emerged all over the body) rat blood vessel was replaced for the one structured at the lower part of kidney.

C: Removed blood vessel chamber (7 days after the transplant, LacZ dyeing); greater omentum bound to transplanted blood vessel chamber (from recipient) invaded media with large number of capillaries (See the arrow in the above figure)

Immediately after the pilot experiment with this small animal, I examined this vascular bed tructuring with pig model as a large animal.



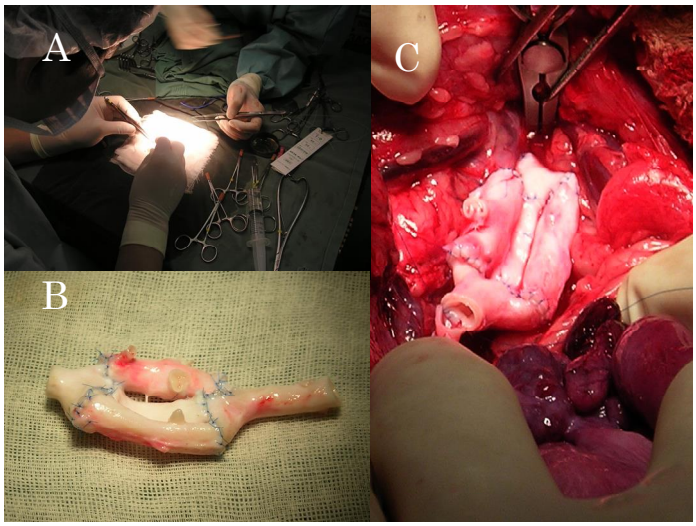
A: Blood vessel chamber with valve from frozen pig; blood vessel chamber with vascular anastomosis between pulmonary artery with valve and ascending aorta. Aimed at decellularization through destruction of pig living cells by freezing.

B: Replacement of arteries by decellralizated blood vessel chamber, exchanged with other matured mini pigsôabdominal aorta in the same position bound with greater omentum around it and encircled with synechia preventive film.

C: Angiography (2 weeks after the transplantation); confirmed of the smooth flow of blood into transplanted blood vessel chamber.

D: Macroscopic evaluation of enucleated specimen; confirmed of blood componentõs composure to the decelluralizated valve.

In addition, experimented the trial to enlarge the size of blood vessel chamber by increasing the number of beating vessels (multiple method).

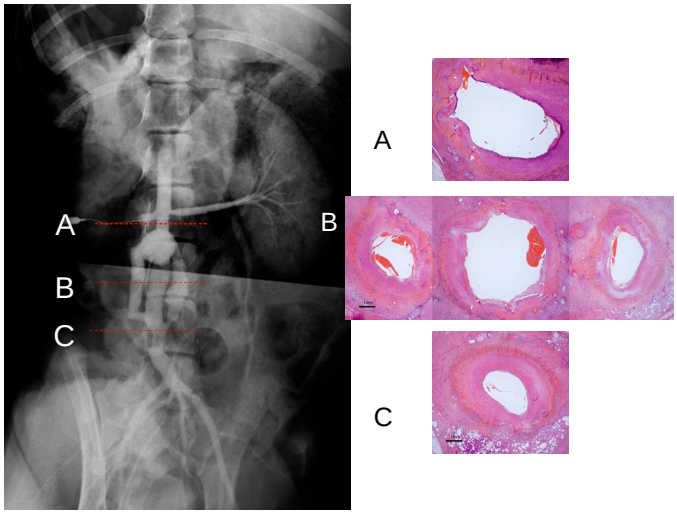


A: Composing surgically the enucleated vessel from the sacrificed pig.

B: Multiple-method blood vessel chamber generated from pig aorta.

C: The replacement of pigsôabdominal aorta in the same position from another pig.

Angiography and isolated blood vessel pathologic images after two weeks from the day operated.

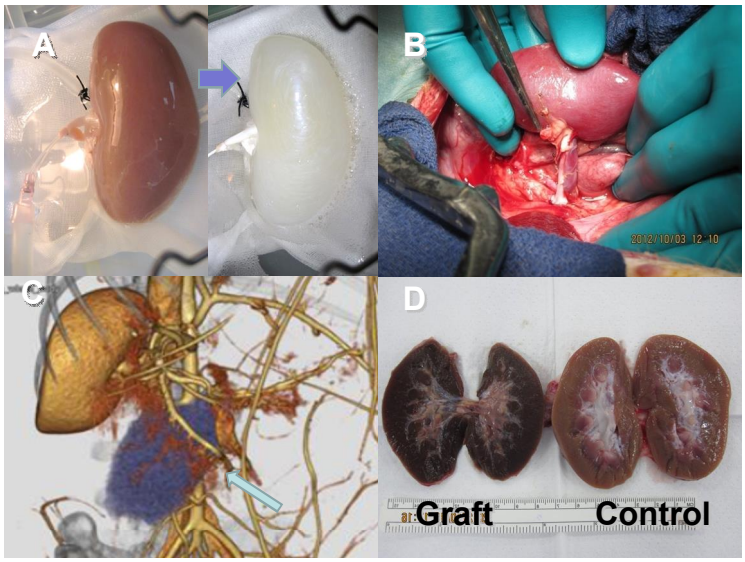


The left figure shows the enucleating points A, B and C with red dotted lines and the right figure shows the pathology specimen. The blood flow of transplanted blood vessel chamber was excellent except the fact that intimal hyperplasia was prominent.

It has been known that the antigenicity of human blood vessel tissue is decreased through decellularization by freezing. The part of cells in large-scale blood vessel is kept remained in the conventional method and it is subject to inflammation (in the year 2008).

The overview for new decellularization technolog

As the conventional decellurlarization by freezing was not sufficient enough, I have been further researching in the related field. In the consequence, I have shifted my focus on the infusion decellularization method of re-frost pig organ with blood vessel in surfactant which was enucleated and once kept frozen.



A: Enucleated pig kidney decellularized

B: Assessment of decellularized kidney graft by transplantation

C: Assessment of blood flow of decellularized graft by contrast-enhanced CT

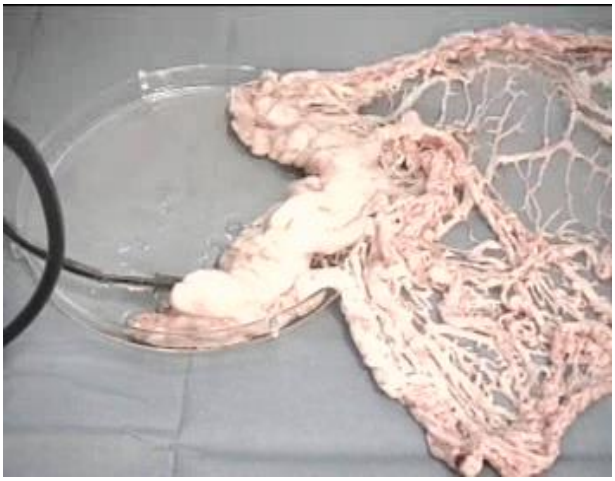
D: Macroscopic comparison with regular kidney

(E. Kobayashi, Kidney and Dialysis 2014)

The decellularized graft is completely free from parenchymal cells. When it is connected to circumfusion culture device, vascular endothelium and parenchymal cells are cultured in antegrade flow to some extent. In near future weâll be in the next step to develop the in-vitro cell-filling technology (in the year 2013).

My contribution to the project

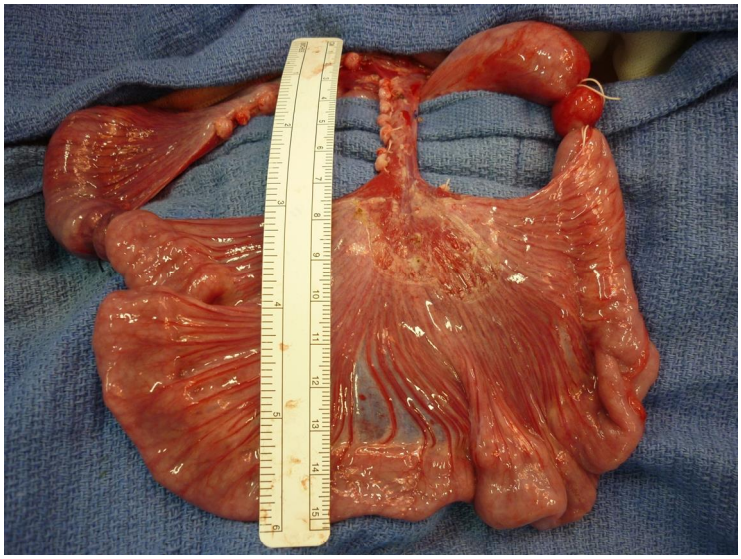
I once reported that the abdominal greater omentum in the process of generating beating blood vessel in rat model was applicable as a blood flow source from the recipient in 2007. Then taking the scale of clinical application into consideration, the greater omentum together with blood vessels have been enucleated from the experimental pig. A series of researches on the blood vascular system have started from here.



The greater omentum capillary plexus graft of pig using stomach greater omentum arteriovenous as inflow and outflow blood vessels

The project is focused on the fabrication of vascular bed by making full use of large blood vessel network and capillary plexus in pigs as tissues and organs with high continuity. In 2014 as a kick-off year, the trial for multiplying of myocardium sheets in human scale by enucleating aseptically the greater omentum accompanied with blood vessel circuit from experimental mini pigs. In the consequence, the adequate decellularization of the greater omentum circuit follows for the purpose of fabricating human vascular endothelium.

On the other hand, I reported in the paper that pig small intestine ñBranched small intestine graftò could be the organ for liver tissue fabrication (Iwasaki J, Organogenesisi 2013). By utilizing this technology I fabricate vascular bed with large-scale inflow blood vessel through the decellularization of segmented small intestine of pigs. Combining it with tube or multilayered methods of myocardium sheets developed by Dr. Shimizu team, we challenge for the 3-D organ fabrication based on human-size myocardium.



The small intestine of pig has a so-to-speak ñstrip-shapedò peripheral artery structure with extremely abundant vascular plexus. The objective is to generate in-vivo vascular bed in the newly installed circumfusion culture unit for segmented small intestine.

The essentials to source the pigsôorgans for medical materials are the ones with traceability not like livestock pigs. Furthermore, these pigs should be kept in the SPF facilities and the organs are to be enucleated as possible as the GMP level. Through the development of this project and my leading position in this research field in our country, we aim at establishing the supply chain to provide pig organs as medical materials first time ever in Japan.