Yamaton K Project in collaboration with Jikei Medical University & Meiji University

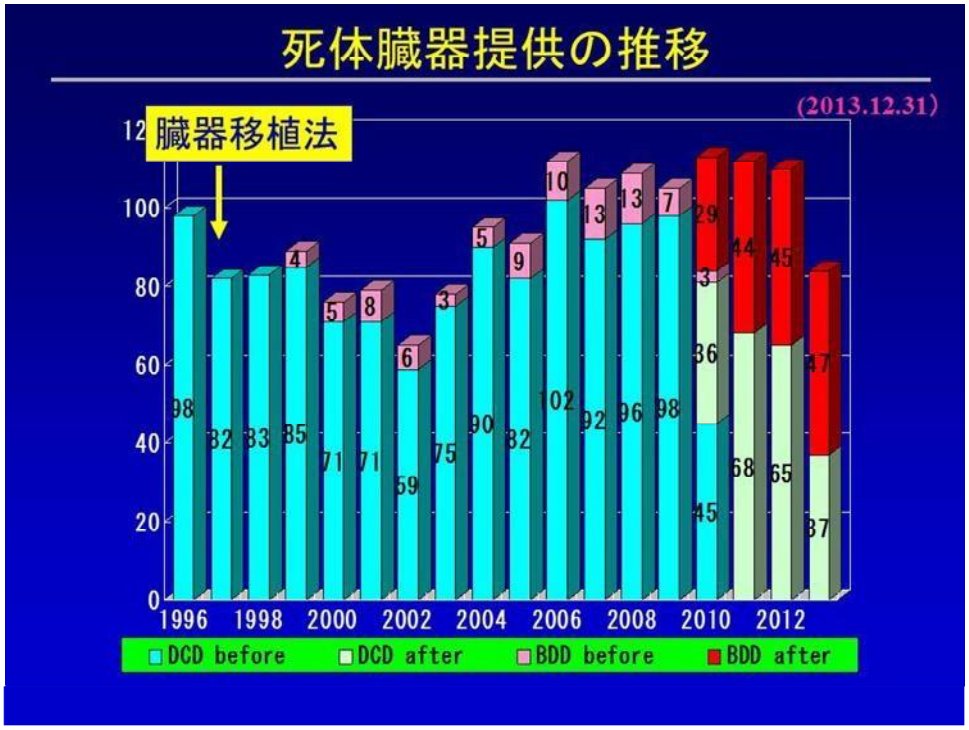
Eiji Kobayashi M.D. Ph.D.
Keio University School of Medicine,
Department of Organ Fabrication

Background

Currently in our country, the therapeutics for end-stage renal failure mainly consist of the following: Hemodialysis, Peritoneum dialysis and Kidney transplantation. Comparing with other countries on global basis, dependence on hemodialysis has been larger and it has been an important issue from medical economy viewpoint. Also kidney transplantation itself has been relying on live donors in most of the cases and there has been a sort of structural problem.

I have done a survey and reported that patients in our country suffering from kidney malfunction have been sometimes involved in unethical transplantation in overseas countries; the fact has been detailed in the special research project entitled "Current situation on medical tourism and risk analysis survey and research" in 2005 by the Ministry of Health, Labor and Welfare. This academic activities mainly in relation with Japan Society for Transplantation in collaboration with The Transplantation Society (TTS) and International Society of Nephrology (ISN) have played an important role to have composed the international Istanbul Declaration against organ trade and medical tourism in 2008 and have impacted greatly on the revision of Japanese organ transplantation law in 2009. However, after these important changes, the drastic decrease in the number of donor after cardiac death (DCD) has been reported while the endeavors to strengthen the safety of live donor and increase in brain-death transplantation have been made (Figure 1).

Therefore, the direction toward which the research is heading is that we need to make our utmost effort to respond to the will of the donor with cardiac arrest from whom the kidney has been enucleated. In other words, no matter what the situation is, we need to make the best effort to transplant it safely.



The best effort to respond to the will of donors with cardiac arrest.

I have been proposing the new methodology to transplant all the kidneys with cardiac arrest as below:

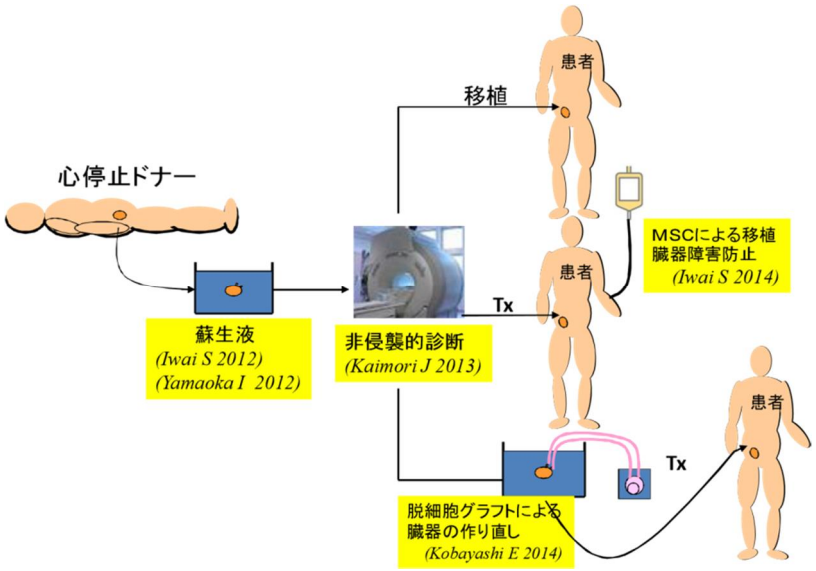


Figure 1: The proposed protocol to enhance the kidney transplantation from the donor with cardiac arrest

There arises the shortage of evolving ATP(adenosine triphosphate) due to the extreme hypoxia in the organs of donors with cardiac arrest. The conventional hypothermic preservation method only acts as delaying the speed of viability of secreted organs but never acts toward improving its vital condition. Recent years in clinical trials the organs with cardiac arrest have been kept at ordinary temperature of 20 degrees Celsius (Hosgood 2011). I have proven that extracellular preservation liquid including trehalose at ordinary temperatures between 20 and 22 degrees Celsius is suitable for rat kidney transplant model (Iwai S, 2012) and adding oxygen by bubbling is noxious due to the emergence of active oxygen and oxygen carrier such as hemoglobin is indispensable (Yamaoka I, 2012). In the consequence we have made it clear that we can judge the prognosis after the transplantation for the candidate kidney in marginal situation through noninvasive diagnostic imaging device such as MRI (Kaimori J, 2013). Furthermore, we have been researching that mesenchymal stem cell addition improves the functionality of transplanted marginal kidney (Iwai S, 2014) and have extended our research to the degree that we create extracellular matrix of kidney with deceased cells through reflux decellelarization method and refill it with newly created kidney cells (Kobayashi E, 2014).

Fabrication of totally new kidney

Over 10 years I have been elaborating the project named “Yamaton K” for the purpose of creating human kidney in collaboration with Prof. Takashi Yokoo (Jikei Medical University) and Prof. Hiroshi Nagashima (Meiji University). Yamato means an ancient name of Japan, ton means pig in Japanese and K means the capital letter of Kidney.

(Team Yamaton K in the year 2014)

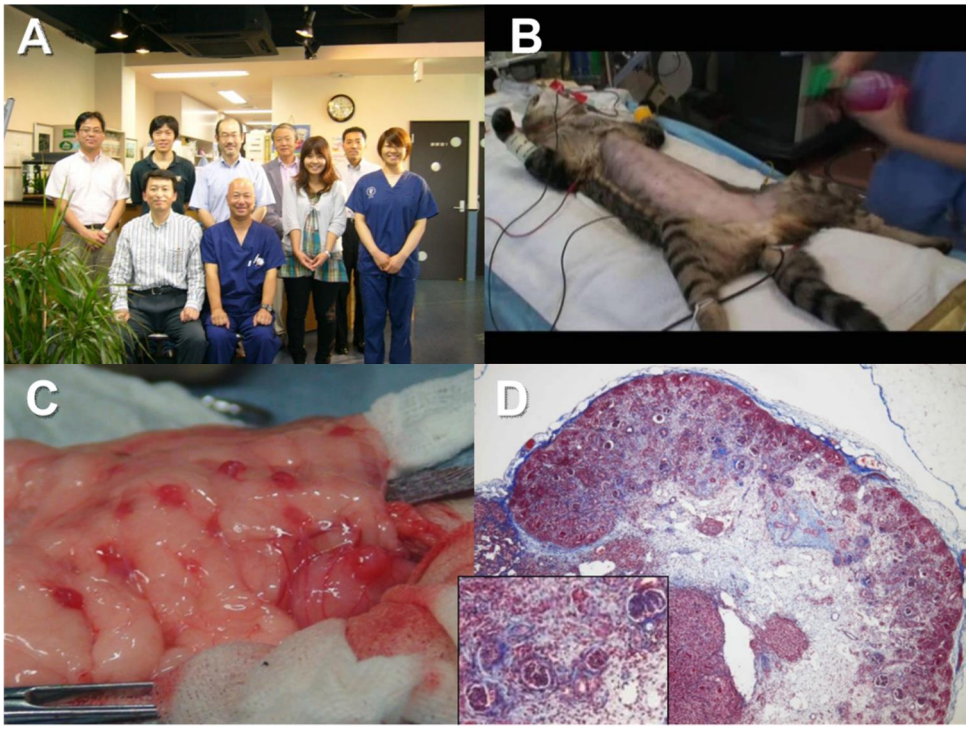
Making full use of the know-how through the acknowledgement of generating fetus organs

The method to understand the principle is shown by the symptom that chimera organ is generated through the injection of human stem cells to fetus animal. It is called “In vivo bioreactor”. In other words, the method is translated to inject human stem cells to xenogeneic animal fetus before the generation of immune function and to induce differentiation of human stem cells to organs in accordance with animal fetus growth process. In the dawn period of the research Prof. Mackenzie has tried to induce differentiation of human mesenchymal stem cells to sheep fetus by way of mother’s uterus (Mackenzie 2001). Prof. Yokoo’s experiment to generate kidney organoid by injecting human mesenchymal stem cell (MSC) to animal fetus has been directly proven by the artificial uterus device (Yokoo T, 2005). Furthermore, in recent years Prof. Hiromitsu Nakauchi (Tokyo University) team has been researching for organ generation to small animals by injecting iPS/ES cells to xenogeneic fertilized egg thorough blastocyst replacement therapy and to complement stem cell gene through the knock-out of gene related to kidney generation (this case Sall 1) in xenogeneic animal (Usui 2012). For now it is up to establish a solution to ethical issues of the method because it requires to generate xenogeneic embryo between animal and human.

In the consequence the therapy to transplant the growing organ bud to the patients is highlighted. It is named “Organ Bud Transplantation”; it represents the method of generating organ bud in vitro and nurture it in the body of patients through transplantation. Prof. Kenji Osafune team (Kyoto University) has generated distal urinary duct from co-cultivation of fetus kidney cells of mouse and intermediate mesodermal cells induced from human iPS cells (Mae, 2013). And also Prof. Ryuichi Nishinakamura team (Kumamoto University) has generated somatic stem cells and kidney precursor cells from human iPS cells and succeeded in creating human glomerulus and renal tubules from direct cultivation with mouse fetus vertebra in conjunction with these iPS-derived cells (Sakaguchi, 2013). Both research results have made kidney development process clearer and also have contributed to 3-D structuring of kidney. This organ bud transplantation methodology started from the research of Prof. Mac Hammerman team (Washington University) who used kidney and pancreas organ buds from pig fetus (Takeda S, 2006).

The challenges to bigger animals by the team Yamaton K

Up to now with Prof. Yokoo team I have gone through non-clinical trials for the purpose of Kidney Bud Transplantation for clinical use. In 2008 the team started experiments of transplanting pig kidney to cat.

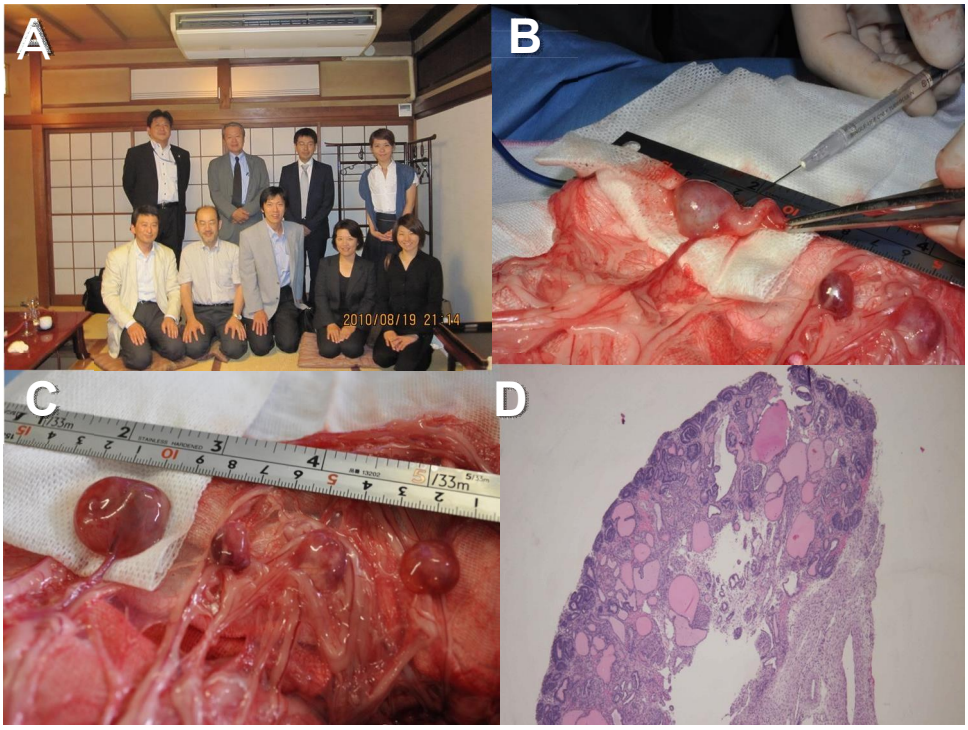


A: Yamaton K team in the year 2008　B: Transplantation of pig kidney to experimental cat

C: The growing kidney within greater omentum of operated cat　D: Transplanted kidney one month after the transplantation

As the application directly to the patients requires the same legal procedures as xenogeneic transplantation, we have rather pushed forward the verification of effectiveness and safety with pet cats.

In 2009 I was appointed to the special counsel for Otsuka Pharmaceutical Factory, Inc. in order to accelerate the research between pig and pig with the large budget which includes outsourcing human resources.



A: Yamaton K team in the year 2010 B, C: The transplanted kidney withholding urine between pig and pig

D: The transplanted kidney with hydronephrosis due to the accumulation of urine

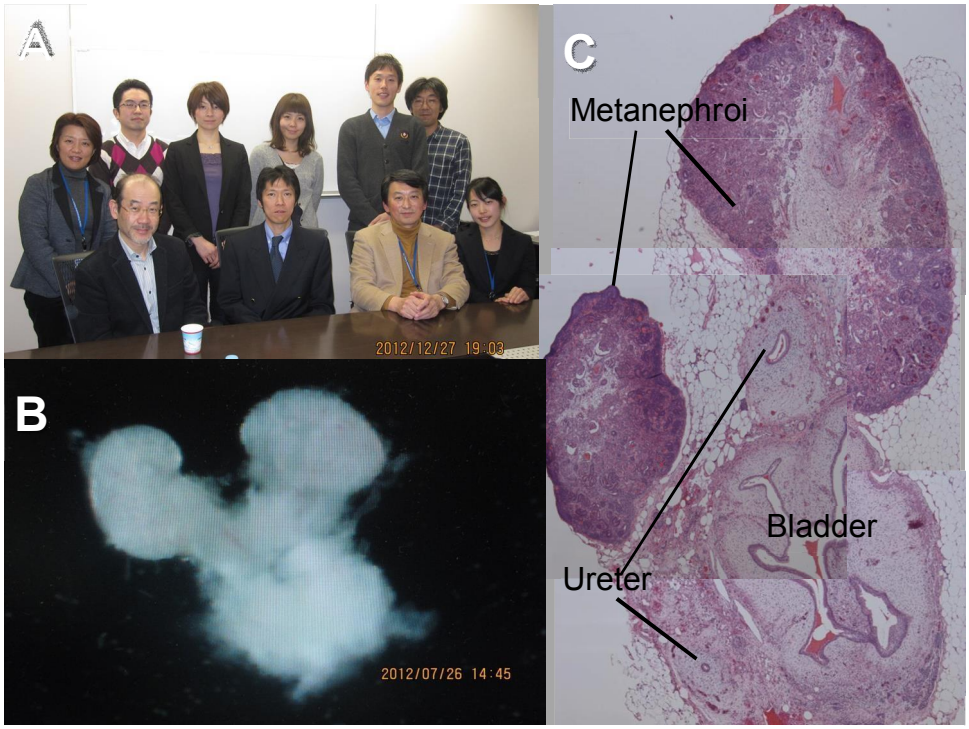
It has been found that immunosuppression gives a chance to enhance kidney growth and consequently, we could generate a transplantable kidney with the function of stretching urinary duct (above Figure B).

Simultaneously we have been researching for the methodology for human chimerization by inserting MSC as human stem cells into animal kidney bud. The pig kidney bud with human MSC has shown the possibility to generate complete human tissue in pig body under the conditions that it temporarily requires for the growth an immunosuppressive agent and the deletion of pig tissues by chemicals in the stage that human stem cells have been induced differentiation to kidney cells to some extent (Matsumoto, 2012).

New Breakthrough

The challenges we should overcome on the above method are how we can structure tissues to reserve temporarily the urine generated from the growing kidney. And also it has been further researched to create a route for the urine to be led outside the body through connecting with the conventional urinary duct. In 2013 we have developed a **cloaca** graft which acts as a combination of bladder and urinary duct in the kidney graft. A **cloaca** in zoological anatomy is known to be the posterior opening that serves as the only such opening for the intestinal, reproductive, and urinary

tracts of certain animal species. The name "**Cloaca Maxima**" derives from Ancient Roman sewer system.



A: Yamaton K team in the years 2012~2013　B: Newly developed pig cloaca graft

C: Pig grown cloaca graft transplanted to cat

We have made further steps toward our dreams of kidney regeneration by utilizing the cloaca graft.