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Original Article

Evaluation of two kinds of materials for whole kidney regeneration

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Abstract: Kidney transplantation is now one of the most effective treatment for end-stage renal failure. However, organ shortage remains a major problem. Hence, an alternative solution for the limited donor organs is in great need. Xenotransplantation of metanephros and cell-scaffold technology (CST) provide new ways for kidney regeneration. The structure and function of regenerated kidneys using these two methods are compared to identify the trend for kidney regeneration. Kidney scaffolds, which were decellularized by SDS perfusion, were recellularized with human umbilical vein endothelial cells (HUVEC) and mouse embryonic stem (ES) cells. Metanephros from embryonic day 13.5 were transplanted into the omentum of adult rats. After cultured in vivo for a period, the structural integrity was evaluated. The quantity and type of cells of kidney generated from metanephros were much more than that of the kidney regenerated from CST. But the latter was more suitable for transplantation. Both kidney regeneration methods have their own advantages, and one method which could offer all these advantages may be the optimal way for kidney transplantation.

Keywords: Stem cells, regenerative materials, kidney regeneration, scaffold, metanephros, transplantation

Introduction

End-stage organ failure is a key challenge for the medical community because of the ageing population and the severe shortage of suitable donor organs available [1]. Chronic kidney disease is a leading cause of mortality and morbidity worldwide, affecting about 8% to 16% of the global adult population [2]. Currently there is no good treatment for end-stage renal disease (ESRD) except dialysis and transplantation. Although the condition could be ameliorated with dialysis, it could not solve the problem that the homeostatic and endocrine functions of the kidney was lost [3-5]. Although kidney transplantation keeps all the functions of the kidney, shortage of donor organs is still the main problem to be solved. In addition, patients who received donor kidneys must tolerate the immunosuppressive medications for life to prevent organ rejection [6, 7]. Consequently, finding of new sources of functional and transplantable kidneys is in extremely urgent need.

Stem cells techniques and regenerative medicine provided the possibility for production of functional kidneys. Many researchers have targeted cell-scaffold therapeutic approaches as the way to produce an alternative kidney for transplantation [8-11]. This method contains two processes: decellularization and recellularization. Decellularization is the process of removing cellular components from the donor tissue using detergents or enzyme [12]. The decelluarized organs, known as scaffolds, maintained the extracellular matrices (ECM) and biologic factors. And intact vascular network was well preserved, which was important for recellularization and transplantation. Then some progenitor cells or adult cells were seeded on the scaffold, and allowed to organize into functional components of the organ, which was called recellularization. Currently, CST has enabled researchers to develop some bioengineered organs, such as lungs [13, 14], heart [15], and liver [16, 17]. And kidney engineering is still in research because of the complex structure and cell types [10, 11, 18].

The metanephros, which constitutes the primordial mammalian kidney, is a promising potential source for the generation of a functional whole kidney [19-22]. The metanephros contains few or no antigen presenting cells because it has no vasculature for several days following the formation of kidney [23]. And the transplanted metanephros would be vascularized in part by blood vessels originating from the host. So this method could partially avoid the immuno-rejection problem. Some researches have been performed to study the renal function of transplanted metanephros [19, 21, 24, 25]. These results indicated that transplantation of metanephros into adult hosts is possible.

These kidney regeneration methods may produce a functional kidney, but each of them has some disadvantages. Studies are carried out on kidney regeneration using these two methods, structure of the regenerated kidney is evaluated, and the trend of kidney regeneration is analyzed.

Materials and methods

Kidney regeneration through CST

Preparation of decellularized kidney scaffolds: For this experiment, we isolated rat kidneys from male Wistar rats (Animal Center of Shandong University, Jinan, China) weighing 220-400 g. All the procedures on these animals were approved by the Animal Ethics Committee of Shandong University (Jinan, China) and the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (NIH Publication No. 85-23, revised 1996) was followed. Kidneys were removed and perfused with some solutions in the following sequence: 0.01 M phosphate buffered saline (PBS, pH 7.4) for 15 min, 0.5% sodium lauryl sulfate (SDS) for 4 h, and then PBS for 24 h to remove SDS.

Characterization of the decellularized scaffold

Histological examination: To assess cell and nuclear clearance as well as preservation of collagen, hematoxylin & eosin (HE) (Solarbio, China) were used after fixation in 10% formalin, paraffin embedding, and sectioning.

Immunohistochemistry for ECM

Tissue slides were fixed in 4% paraformaldeyhyde and then processed using the common protocol of immunohistochemistry. The following primary antibodies were used: mouse monoclonal anti-Fibronectin antibody (Abcam, Cambridge, MA, USA, ab194395, 1:200), and rabbit polyclonal Anti-Collagen IV antibody (Abcam, ab6586, 1:200).

Imaging of the vasculature

To confirm the integrity of the vascular tree, x-ray fluoroscopy was performed on the bench before implantation using a Siemens SIREMOBIL Compact L C-arm. Conray (iothalamate meglumine) (Mallinckrodt Inc, St. Louis, MO) contrast agent was diluted at 1:50 in distilled water and perfused through the vasculature at a rate of 0.2 mL/minute.

DNA and collagen quantification

The DNA content of fresh and decellularized kidney was quantified using the tissue DNA isolation kit (PureLink Genomic DNA MiniKit, Invitrogen) according to the manufacturer's instructions. Optical densities at 260 nm and 280 nm were used to evaluate the purity and yield of nucleic acids, which was quantified on the basis of 280 nm absorbance.

Soluble collagen was quantified using the Sircol Assay (Biocolor), as per the manufacturer's instructions. All concentrations were determined on the basis of a standard curve generated in parallel, and values were normalized to the original tissue dry weight.

HUVEC and mES cell culture

We performed primary culture of HUVEC and expanded them at passages 8-10 in ECM (sciencell). At the time of cell seeding, cells were centrifuged and resuspended in 3.0 ml of ECM, and the concentration was 1.0×10^7/mL.

mES cells were cultured and passaged every two days on mitotically inactivated mouse embryonic fibroblast (MEFs) in proliferation medium DMEM supplemented with 1 mM sodium pyruvate, 100 mM nonessential amino acids, 2 mM L-glutamine, 100 U/mL penicillin-100 mg/mL streptomycin, 0.1 mM mercaptoethanol, and 10% ES cell qualified FBS. The preparation for cell seeding was the same as that of HUVEC.

Recellularization of kidney scaffolds with HUVEC and mES cells

After decellularization and sterilization, the scaffolds were seeded with 1×10^7/mL HUVEC

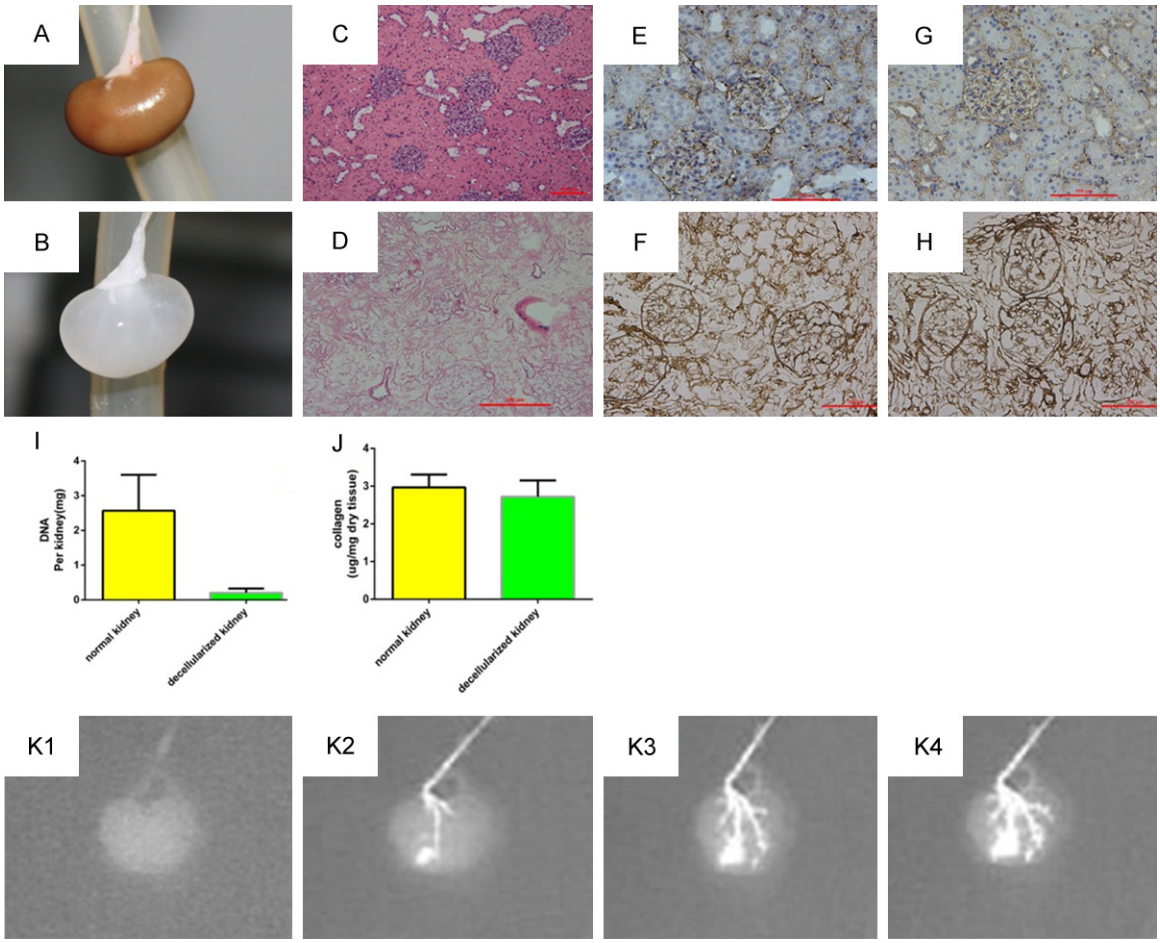

Figure 1. The production of kidney scaffold. A, C, E, G: Shows the characteristics of normal kidney, while B, D, F, G: Shows the characteristics of the kidney scaffold. A and B: Gross feature of the kidney and kidney scaffold. After decellularization, the kidney became semi-transparent. C and D: HE staining shows that only collagen was left on the scaffold after decellularization. E-G: Immunochemistry shows that collagen IV and fibronectin was well preserved after decellularization. I: DNA content was almost removed after decellularization. J: Before and after decellularization, there was no obvious changes for the content of collagen. K1-4: x-ray fluoroscopy shows that the vascular system was well preserved after decellularization.

diluted in 2 ml medium from the renal artery and the same content of mES cells through the ureter. Cells were allowed to attach overnight, after which perfusion of culture was resumed. Perfusion media was infused through sterile access ports (Millipore) to minimize the risk of contamination. Media was allowed to equilibrate with 5% CO_2 and 95% room air before reaching the cannulated renal artery at 1 mL/min for 7 days.

Kidney regeneration by transplantation of metanephros

Metanephroi retrieval and transplantation: We isolated metanephros for this experiment from embryonic day 13.5 (E13.5) Wistar rats (Animal Center of Shandong University, Jinan, China) using the microsurgery techniques. Then the metanephros was placed immediately into ice-cold Ham's F12 DMEM (DMEM: F12) solution (Conning).

After isolation, the metanephros was immediately transplanted in the omentum of two-month-old female Wista (host) rats. The host rats had their left kidney removed. And then the incision was closed. Some transplanted metanephroi was removed after six weeks following transplantation.

Characterization of the transplanted metanephroi

Histological examination: To assess the histology of the transplanted metanephroi, hematoxylin & eosin and Masson Trichrome staining

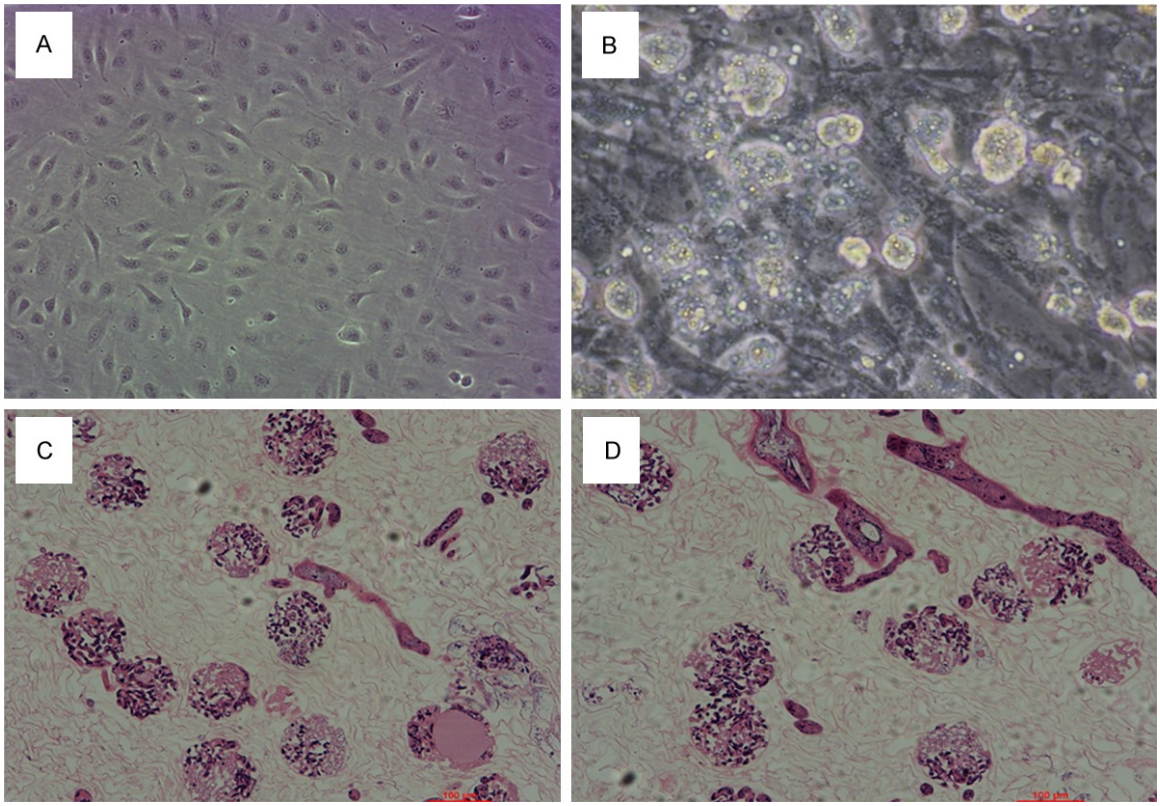

Figure 2. Recellularization of the kidney scaffold. A: HUVEC. B: mES cells. C and D: HE staining shows that the cells we seeded attached to the kidney scaffold.

(Solarbio, China) were used after fixation in 10% formalin, paraffin embedding, and sectioning.

Immunohistochemistry for metanephro

The E13.5 and transplanted metanephroi was fixed in 4% paraformaldeyhyde, quenched for endogenous peroxidase in 0.3% Hydrogen Peroxide, blocked with 4% normal goat serum in PBS and incubated with primary antibodies for 24 h at 4°C. They were incubated with biotinylated secondary antibodies for 30 min at room temperature. The following primary antibodies were used: rabbit polyclonal to Nanog (Abcam, ab106465), rabbit polyclonal to SOX2 (Abcam, ab97959), rabbit polyclonal to OCT4 (Abcam, ab18976), rabbit monoclonal to pax-2 (Abcam, ab79389).

Statistical analysis

Quantitative results were reported as mean standard deviation. Student t-test was used to reveal differences in the level of cytokines, DNA and collagen content among different groups. The significance level was set at 0.05 and Graphpad prism 6.0 was used for the analyses.

Results

Characterization of the decellularized scaffold

Our procedures successfully decelluylarized rat kidneys as shown by a near transparent appearance (**Figure 1B**), and H&E staining showed a lack of cellular nuclear material with only collagen left (**Figure 1D**).

The ECM of the decellularized renal scaffold, was preserved as seen by the distribution of specific ECM proteins, collagen IV and fibronectin, which were similar to that in native rat kidney tissue (**Figure 1F** and **1H**).

We removed most DNA for comparison to the native organ (**Figure 1I**), while retaining total collagen levels similar to those in cadaveric kidney tissue (**Figure 1J**).

Although the kidney was well decellularized, the intact vascular network maintained the hierar-

chical branching structures through the renal artery as depicted with contrast media (**Figure 1K**).

Recellularization of the decellularized kidney scaffold

We recellularized the scaffold with HUVEC (**Figure 2A**) and mES cells (**Figure 2B**).

After decellularization and perfusion with culture medium, these cells were able to attach and grow in the ECM (**Figure 2C** and **2D**).

Throughout the entire test period after in vivo transplantation, regenerated kidney grafts appeared well perfused without any evidence of bleeding from the vasculature. Regenerated kidneys produced urine shortly after removal of the vascular clamps.

Characterization of metanephros

Metanephros was retrieved successfully by micro-surgery (**Figure 3A** and **3B**).

Six weeks after transplantation of metanephros in the omentum, we performed immunochemistry to detect markers of kidney. H&E staining showed that nephron was formed, which contained glomeroli and renal tubule (**Figure 3C** and **3D**). The markers of stem cells, such as OCT4, NANOG and SOX2 were negative (**Figure 3E-G**), which demonstrated that the cells of metanephros have differentiated to other cells. And the key marker of kidney development, PAX2 (**Figure 3H**), was positive, which showed that the metanephros we transplanted had differentiated to renal cell linage. The vascular marker, CD31 (**Figure 3I**) was also positive.

Discussion

Whole kidney regeneration holds great promise for ESRD patients. There are several ways for kidney regeneration, transplantation of metanephroi and cell-scaffold techniques was two of them. In the past few years, several groups have tried these ways for kidney regeneration [10, 11, 18, 19, 21, 22]. The aim of this study was to apply these two techniques in rodent model, and compare their advantages and disadvantages in structure and function of the regenerated kidneys.

Methods of CST have varied between different researches. The main goal was to produce a scaffold without disrupting its ultrastructure and improve the cell attachment in the scaffold. We adopted the basic criteria for scaffold, which included: (1) Remove native cellular materials (DNA content at or below 50 ng DNA/mg tissue) [26]. (2) Preserve native extracellular components and ultrastructure of the decellularized organ [17, 27, 28]. (3) Preserve the intact vascular system.

We have developed an effective and fast method for kidney regeneration. The vascular trees of decellularized renal scaffold were intact at all hierarchical levels. The collagen of ECM was well preserved and Residual SDS levels in the scaffolds were negligible. The decellularized kidney scaffold was non-cytotoxic and promoted cell attachment and differentiation when seeded with ES cells. There were several protocols to produce decellularized scaffold, which may take several hours, days or even weeks to decellularize the kidney [10, 18, 29, 30]. The longer the time is taken , the more serious disruption for ECM micro-structure and cytokines occurs. We demonstrated that complete decellularization of rat kidneys can be safely and effectively achieved using SDS alone, as documented by the histologic and immunochemistry findings which confirmed complete cell removal without loss of structural ECM proteins.

Another key step for CST was techniques of recellularization. Several groups of researchers are working on this step in rodent models [10, 11, 18]. But there are still many aspects for improvement. In our opinion, stem cells are the ideal cell type for kidney regeneration. Song et al. reported that recellularization with endothelial cells and neonatal kidney cells resulted in a functional kidney [10]. But the time required for maturation of the seeded cells is not clear and immuno-rejection problem should not be ignored. Ross et al. and Bonandrini et al. reported that recellularizing the scaffold with mES cells leaded to a regenerated kidney. But some problems were encountered in the process of recellularization. The cells attached to the scaffold did not grow well and no function was described in these two studies. In our study, the cells seeded on the scaffold grew well after recellularization and perfusion with the self-made whole kidney culture system. And the

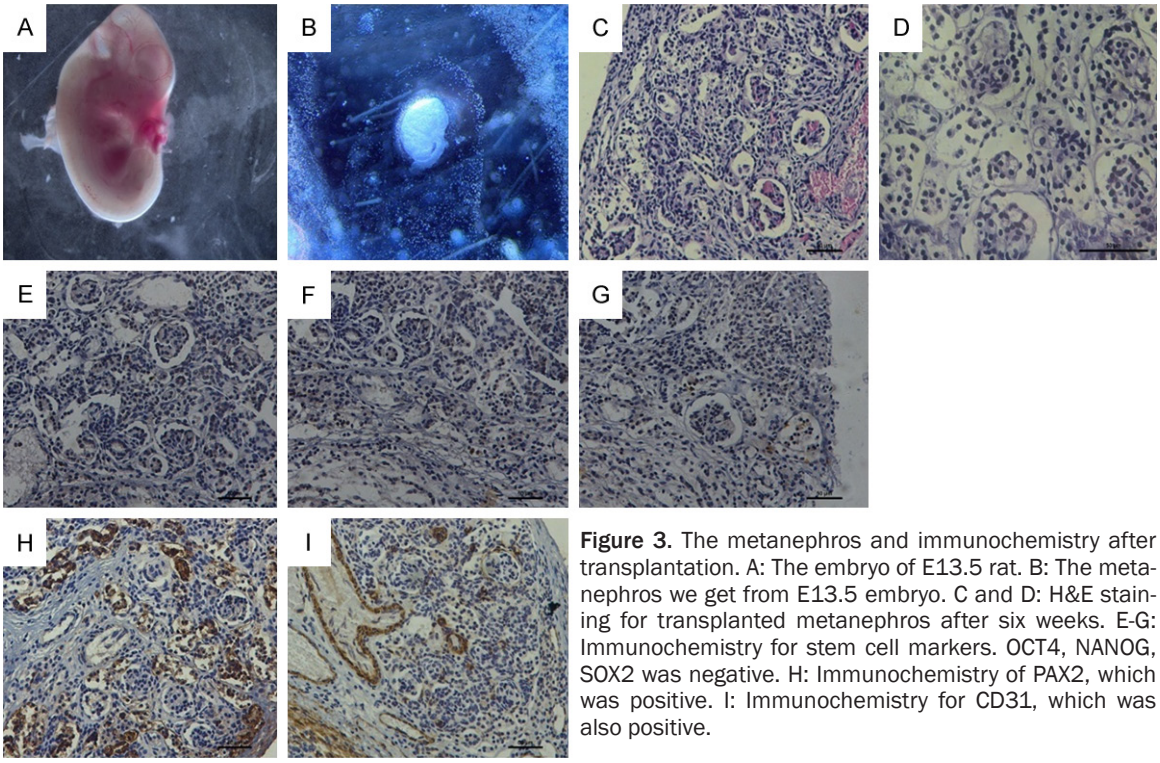

Figure 3. The metanephros and immunochemistry after transplantation. A: The embryo of E13.5 rat. B: The metanephros we get from E13.5 embryo. C and D: H&E staining for transplanted metanephros after six weeks. E-G: Immunochemistry for stem cell markers. OCT4, NANOG, SOX2 was negative. H: Immunochemistry of PAX2, which was positive. I: Immunochemistry for CD31, which was also positive.

regenerated kidney could be transplantable and functional.

It has been known for over four decades that embryonic tissues are less immunogenic compared with their adult counterparts [31]. Thus we chose the earliest time (E13.5) when the metanephroi was more complete and least prone to immune rejection. Our study shows long-time survival of metanephros transplanted to the omentum and kidney formation.

Omentum is an immunological site for xeno- and allo-transplantation [32]. So it's inevitable to have immune response to transplantation a kidney from one rat to another [33]. Our study shows that metanephros from E13.5 did not lead to immune response. Abrahamson et al. transplanted metanephros from E17 rats [34] and Roger et al. from E15 rats [19]. These studies showed that E17 and E15 metanephros were more immunogenic than E13.5 metanephros. There are two reasons which may explain this phenomenon: (1) unlike a developed kidney, the E13.5 metanephros is a non-vascularized organ. After transplantation, the vascular system from the receptors grows into the metanephros; (2) the earlier we get metanephros from the embryos, the less immune response occurs; the early kidney precursors possibly lack mature antigen-presenting cells (APC) [22, 35].

In this study, we performed two methods for kidney regeneration. The metanephros transplanted to the omentum could form the nephron and the PAX2 genes could be expressed. And the kidney produced from this method was less immunogenic. But there are two main shortcomings for this method (1) the kidney obtained is not transplantable, because the vascular system was complex; (2) the ureter is not very clear and it should be connected with the receptor's ureter or bladder. While the CST preserved the 3D structure of the normal kidney, which makes it easy for transplantation. But the kidney function is limited because the recellularization techniques is being improved. In the future, we should make more efforts in combining the advantages of these two methods to produce a functional and transplantable kidney.

Conclusion

In this study, we tried two methods for kidney regeneration. Both methods could develop a kidney and had different advantages. In the future, we should make more efforts in combin-

ing the advantages of these two methods to produce a functional and transplantable kidney.

Acknowledgements

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Disclosure of conflict of interest

None.

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References

[1] Badylak SF, Weiss DJ, Caplan A and Macchiarini P. Engineered whole organs and complex tissues. Lancet 2012; 379: 943-952.

[2] Jha V, Garcia-Garcia G, Iseki K, Li Z, Naicker S, Plattner B, Saran R, Wang AY and Yang CW. Chronic kidney disease: global dimension and perspectives. Lancet 2013; 382: 260-272.

[3] Steer CB, Marx GM, Galani E, Harper PG and Khayat D. Quality of life: it's never too late. J Clin Oncol 2002; 20: 2915-2917.

[4] Ikizler TA. CKD classification: time to move beyond KDOQI. J Am Soc Nephrol 2009; 20: 929-930.

[5] Wolfe RA, Ashby VB, Milford EL, Ojo AO, Ettenger RE, Agodoa LY, Held PJ and Port FK. Comparison of mortality in all patients on dialysis, patients on dialysis awaiting transplantation, and recipients of a first cadaveric transplant. N Engl J Med 1999; 341: 1725-1730.

[6] Luan FL, Steffick DE and Ojo AO. Steroid-free maintenance immunosuppression in kidney transplantation: is it time to consider it as a standard therapy? Kidney Int 2009; 76: 825-830.

[7] Porrini E, Moreno JM, Osuna A, Benitez R, Lampreabe I, Diaz JM, Silva I, Dominguez R, Gonzalez-Cotorruelo J, Bayes B, Lauzurica R, Ibernon M, Moreso F, Delgado P and Torres A. Prediabetes in patients receiving tacrolimus in the first year after kidney transplantation: a prospective and multicenter study. Transplantation 2008; 85: 1133-1138.

[8] Sullivan DC, Mirmalek-Sani SH, Deegan DB, Baptista PM, Aboushwareb T, Atala A and Yoo JJ. Decellularization methods of porcine kidneys for whole organ engineering using a high-throughput system. Biomaterials 2012; 33: 7756-7764.

[9] Guan Y, Liu S, Liu Y, Sun C, Cheng G, Luan Y, Li K, Wang J, Xie X and Zhao S. Porcine kidneys as a source of ECM scaffold for kidney regeneration. Mater Sci Eng C Mater Biol Appl 2015; 56: 451-456.

[10] Song JJ, Guyette JP, Gilpin SE, Gonzalez G, Vacanti JP and Ott HC. Regeneration and experimental orthotopic transplantation of a bioengineered kidney. Nat Med 2013; 19: 646-651.

[11] Ross EA, Williams MJ, Hamazaki T, Terada N, Clapp WL, Adin C, Ellison GW, Jorgensen M and Batich CD. Embryonic stem cells proliferate and differentiate when seeded into kidney scaffolds. J Am Soc Nephrol 2009; 20: 2338-2347.

[12] Baptista PM, Orlando G, Mirmalek-Sani SH, Siddiqui M, Atala A and Soker S. Whole organ decellularization - a tool for bioscaffold fabrication and organ bioengineering. Conf Proc IEEE Eng Med Biol Soc 2009; 2009: 6526-6529.

[13] Petersen TH, Calle EA, Zhao L, Lee EJ, Gui L, Raredon MB, Gavrilov K, Yi T, Zhuang ZW, Breuer C, Herzog E and Niklason LE. Tissue-engineered lungs for in vivo implantation. Science 2010; 329: 538-541.

[14] Ott HC, Clippinger B, Conrad C, Schuetz C, Pomerantseva I, Ikonomou L, Kotton D and Vacanti JP. Regeneration and orthotopic transplantation of a bioartificial lung. Nat Med 2010; 16: 927-933.

[15] Ott HC, Matthiesen TS, Goh SK, Black LD, Kren SM, Netoff TI and Taylor DA. Perfusion-decellularized matrix: using nature's platform

to engineer a bioartificial heart. Nat Med 2008; 14: 213-221.
[16] Uygun BE, Soto-Gutierrez A, Yagi H, Izamis ML, Guzzardi MA, Shulman C, Milwid J, Kobayashi N, Tilles A, Berthiaume F, Hertl M, Nahmias Y, Yarmush ML and Uygun K. Organ reengineering through development of a transplantable recellularized liver graft using decellularized liver matrix. Nat Med 2010; 16: 814-820.
[17] Baptista PM, Siddiqui MM, Lozier G, Rodriguez SR, Atala A and Soker S. The use of whole organ decellularization for the generation of a vascularized liver organoid. Hepatology 2011; 53: 604-617.
[18] Bonandrini B, Figliuzzi M, Papadimou E, Morigi M, Perico N, Casiraghi F, Dipl C, Sangalli F, Conti S, Benigni A, Remuzzi A and Remuzzi G. Recellularization of well-preserved acellular kidney scaffold using embryonic stem cells. Tissue Eng Part A 2014; 20: 1486-1498.
[19] Rogers SA, Lowell JA, Hammerman NA and Hammerman MR. Transplantation of developing metanephroi into adult rats. Kidney Int 1998; 54: 27-37.
[20] Matsumoto K, Yokoo T, Yokote S, Utsunomiya Y, Ohashi T and Hosoya T. Functional development of a transplanted embryonic kidney: effect of transplantation site. J Nephrol 2012; 25: 50-55.
[21] Rogers SA, Talcott M and Hammerman MR. Transplantation of pig metanephroi. ASAIO J 2003; 49: 48-52.
[22] Dekel B, Burakova T, Arditti FD, Reich-Zeliger S, Milstein O, Aviel-Ronen S, Rechavi G, Friedman N, Kaminski N, Passwell JH and Reisner Y. Human and porcine early kidney precursors as a new source for transplantation. Nat Med 2003; 9: 53-60.
[23] Saxen L and Sariola H. Early organogenesis of the kidney. Pediatr Nephrol 1987; 1: 385-392.
[24] Woolf AS, Palmer SJ, Snow ML and Fine LG. Creation of a functioning chimeric mammalian kidney. Kidney Int 1990; 38: 991-997.
[25] Barakat TI and Harrison RG. The capacity of fetal and neonatal renal tissues to regenerate and differentiate in a heterotopic allogeneic subcutaneous tissue site in the rat. J Anat 1971; 110: 393-407.
[26] Crapo PM, Gilbert TW and Badylak SF. An overview of tissue and whole organ decellularization processes. Biomaterials 2011; 32: 3233-3243.
[27] Gillies AR, Smith LR, Lieber RL and Varghese S. Method for decellularizing skeletal muscle without detergents or proteolytic enzymes. Tissue Eng Part C Methods 2011; 17: 383-389.
[28] Badylak SF, Freytes DO and Gilbert TW. Extracellular matrix as a biological scaffold material: Structure and function. Acta Biomater 2009; 5: 1-13.
[29] Ross EA, Abrahamson DR, St John P, Clapp WL, Williams MJ, Terada N, Hamazaki T, Ellison GW, Batich CD. Mouse stem cells seeded into decellularized rat kidney scaffolds endothelialize and remodel basement membranes. Organogenesis 2012; 8: 49-55.
[30] Yu YL, Shao YK, Ding YQ, Lin KZ, Chen B, Zhang HZ, Zhao LN, Wang ZB, Zhang JS, Tang ML and Mei J. Decellularized kidney scaffold-mediated renal regeneration. Biomaterials 2014; 35: 6822-6828.
[31] Medawar PB. Some immunological and endocrinological problems raised by the evolution of viviparity in vertebrates. Symp Soc Exp Biol 1953.
[32] Streilein JW. Unraveling immune privilege. Science 1995; 270: 1158-1159.
[33] Churchill M, Kline R, Schwartz M, Bidani A and Churchill P. Kidney transplants in cyclosporine-treated Sprague-Dawley rats. Transplantation 1990; 49: 8-13.
[34] Abrahamson DR, St John PL, Pillion DJ, Tucker DC. Glomerular development in intraocular and intrarenal grafts of fetal kidneys. Lab Invest 1991; 64: 629-639.
[35] Lacy PE, Davie JM and Finke EH. Prolongation of islet xenograft survival (rat to mouse). Diabetes 1981; 30: 285-291.