IJKD KIDNEY DISEASES

Bioengineered Kidney, Still a Long Way to Go

Alireza Heidary Rouchi

Liver Transplantation Research Center, Imam Khomeini Hospital Complex, Tehran University of Medical Sciences, Tehran, Iran

Keywords. regenerative medicine, kidney transplantation, tissue engineering

Regenerative medicine has attempted to raise hopes to replace failing solid organs as a part of its wide-spectrum mission over the last couple of decades. To generate and implant an organ, taking the global challenge of organ shortage and histological complexity into consideration, has made this endeavor more taxing and strenuous in comparison with other subjects including cells and tissues. As the most prevalent transplanting organ worldwide, to make a kidney in laboratories using decellularized discarded donated kidney as a scaffold and recullalarize that with recipient's native cells to circumvent another unavoidable obstacle, immunological mismatch, has remained a not yet attainable promise. Diversity of cell types and structural complexity of kidney with a range of functions has slowed down the pace of taking steps to fulfill this outstanding ambition, and, accordingly, it would make sense for the nonce that main attempts in limited-funding settings shall be directed to procure more allogenic grafts to partially meet the daily-expanding demands.

IJKD 2021;15:238-41
www.ijkd.org

Brief Comunication

INTRODUCTION

Increasing life-expectancy and increased prevalence of obesity, hypertension and diabetes mellitus, in addition to unhealthy life-style cause increasing number of patients with burdensome End-stage Renal Disease (ESRD) in all countries. End-stage renal disease even with costly Renal Replacement Therapies (RRTs) shortens life-span and causes loosing of disability-adjusted life-years. Among RRTs, renal transplantation provides the suffered patients with the best outcomes; nonetheless, the number of optimal and marginal kidney allografts has not yet met the ever-increasing need.[1-5]

In the context of renal transplantation, ways out of two dilemmas, organ shortage and immunological mismatch, have not yet been found. These predicaments have brought to the attention of researchers in the field of regenerative medicine to find an ideal way to cope with this tough contest. This dream can hopefully comes true once renal transplantation makes possible with a bioengineered and fully immunologically matched graft.[6]

Excluding bio-printing technology, this review aimed to offer briefly the latest tries and achievements in the field of kidney tissue engineering and availability of a renal graft with no worries of rejection and adverse effects of long-life immune-suppression.

EVOLUTION OVER TIME

To make a bio-artificial kidney, to replace filtration process among multiple functions of a non-failing kidney, has been the first concern. So, artificial membranes have been firstly the center of attention in early experiments. In line with this, the researchers have produced membranes with nano-scale pores. In one of those approaches, using silk porous scaffold containing collagen-matrigel, mouse embryonic kidney cells were cultured on and after two weeks in bioreactor culture, a structure partly similar to kidney was developed.[7, 8]

In accordance with several and similar studies, it was widely believed that to construct a bio-hybrid

kidney, two fundamental elements are selection of an appropriate cell population and presence of a supporting scaffold mimicking glomerular and collecting architecture of a normal kidney.[9,10]

Thereafter, small-sized and automated dialysis, applying micromechanics, was come to the minds, combining culture of renal cells and membrane-based filtration system.[11]

The next attempts were focused on using decellularized discarded human kidney which provides the most suitable structure and making site-specific differentiation possible.[12, 13] As a consensus, it is generally agreed that a combination of both natural kidney scaffold and nanoengineered membranes for filtration is the core strategy in the most currently run experiments. In this platform, it is targeted to repopulate the Extra-Cellular Matrix (ECM) of an acellularized allogenic kidney with different autologous or stem cell types. In this method, the immunogenic cellular components are fully removed while its biomechanical integrity and inductive feature are maintained.

HUMAN RENAL SCAFFOLD

Discarded Kidneys

The main source of kidneys for decellularization and preparing an appropriate scaffold is kidneys from heart-beating or non-heart-beating deceased donors whose kidneys are not optimal or even marginal for implantation and in case of prior informed consent for research, from donor's next-of-kin, are offered for decellularization. Annually, a remarkable number of kidneys, procured from deceased donors, are discarded due to different reasons and this figure is increasing steadily, whereas, this rate increased from 20% in 2015 to 30.3% in 2019 in the United States.[14,15] The number represents the proportion of kidneys not used for transplantation to the number of actual heart-beating brain-dead organ donors multiplied by two. To apply the same methodology, the rate in the United Kingdom, Germany and Iran was 20.5%, 13.5%, and 37%, respectively.[15]

Decellularization

Decellularization of whole human kidney has been achieved through perfusion with different concentrations and combinations of Sodium Dodecyl Sulfate (SDS), Triton-X-100, Trypsin, and Deoxyribonuclease (DNase) through renal artery and ureter.[16] In the process of decellularization, and following the cell-scaffold idea, it is aimed to remove cellular structures, debries and at the same time preserving other structures including vascular, cortical and medullary architecture, and collecting system. Meanwhile, in renal ECM, the expression of growth factors shall be remained in order to provoke migration, cell adhesion, proliferation and angiogenesis.[13,16,17]

The steps of decellularization are listed as follow:[16,18]

1. Insertion of angiocatheter in the renal artery and in the ureter
2. Rinsing the kidney via renal artery by distilled water or pump infusion of phosphate Buffer Saline (PBS) at the rate of 12 mL/min for 12 hours
3. Pump infusion of the kidney by 0.5% SDS-based solution at the same flow for 48 hours in both renal artery and ureter
4. Rinsing the kidney with DNase for 6 hours at a flow rate of 6 mL/min
5. Rinsing the kidney with PBS for five days

In this method, all cells and nucleic debries are totally removed and these changes to such a non-immunogenic ECM are assessed by histology, immune-histochemistry, and immune-staining studies. The assessment is completed by scanning electron microscopy, DNA quantitation, glycosaminoglycan and quantification, and vessel morphometry. To assess the integrity of vasculature, the pressure measurement is performed by perfusing the kidney with water through renal artery at the flow rate of 10 to 30 mL/min by inserting a pressure probe into the renal artery.

It is widely established that the human kidney ECM has all the required components and functional architecture by retaining necessary growth factors. This framework induces cell migration, proliferation, differentiation and preserving cell type-specific phenotypes. This matrix is supposed to be slowly degraded and replaced with ECM proteins secreted by growing cells.[16-18]

RECELLULARIZATION

Cell Choices

Although the decellularized kidney has been finalized to be the scaffold of choice to generate a bioengineered kidney, recellularization of the whole kidney ECM has been rarely effective due

mainly to uncertainty about the ideal cell types to make the cellular seeded scaffold functioning. The diversity of cell types of a kidney with more than 26 specialized cell types makes this scenario more complicated. Different cells including renal progenitor cells (embryonic and bone marrow stem cells), renal epithelial and endothelial cells, and Induced Pluripotent Stem Cells (iPSCs) have been used in different trials. It is expected that these cells could generate tubular and endothelial-like structures on the decellularized kidney ECM with preserved architecture.[19-24]

In order to generate a non-immunogenic graft and also to exclude the ethical concerns, the cells should be derived from the recipient. In this concern, iPSCs derived from recipient somatic cells have the capacity of self-renewal and differentiation similar to embryonic stem cells.

Recellularization Techniques

1. Perfusion Technique: This method is considered as the most appropriate seeding approach. The whole decellularized kidney is perfused under high pressure with cell suspension (endothelial and epithelial cells derived from iPSCs) via vascular and collecting system and then the scaffold is cultured in a device named bioreactor.[21,25-27] The pitfall of this technique is incomplete repopulation meaning that the number of cells and formed structures are less than a normal organ.

2. Multiple Injection Technique: Another approach for cell seeding is to inject cells into the cortical region of the kidney parenchyma. So far, this experiment is carried out on animal models. This method provides better parenchymal repopulation, but the endothelialization of kidney vascular system cannot be achieved. [28]

FIRST, *IN VITRO* RECELLULARIZATION

In vivo successful implantation of a bioengineered kidney in animal model has been limitedly reported.[27, 29] Initial animal studies showed that *in vivo* renal decellularized scaffold was not repopulated by host cells unless *in vitro* recellularization is carried out primarily. In this direction, multiple growth factors including epidermal growth factor, transforming growth factor-alpha, insulin-like growth factor, and vascular endothelial growth factor have been successfully used to induce renal cell proliferation and regeneration.[22, 30, 31]

CONCLUSION

This review tries to summarize the latest approaches and achievements in the context of generating bioengineered kidney, taking the long-lasting and ever-growing shortage of renal allografts into consideration. Although the most appropriate scaffold, decellularized human kidney, is available right now, to make a completely seeded scaffold with multiple cell lines and generating a functioning graft still seems a matter of big concern. The research in such fields is extremely fund-consuming and never replaces the establishment of a well-structured organ procurement program from deceased and living donors in low- to medium-income countries, at least for coming decades.

CONFLICT OF INTEREST

The author declares that there are no competing interests.

REFERENCES

1. GBD Chronic Kidney Disease Collaboration. Global, regional and national burden of chronic kidney disease, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet 2020; 395: 709-733.
2. Crews DC, Bello AK, Saadi G, for the World Kidney Day Steering Committee. Burden, Access, and Disparities in Kidney Disease. IJKD 2019;13:1-9.
3. Moradpour A, Hadian M, Tavakkoli M. Economic Evaluation of End Stage Renal Disease Treatments in Iran. Clinical Epidemiology and Global Health 2020; 8: 199-204.
4. Heidary Rouchi A, Mahdavi-Mazdeh M. When is Transplantation with a Marginal Kidney Justifiable? Ann Transplant 2016; 21: 463-468.
5. Nafar M, Aghighi M, Dalili N, Alipour Abedi B. Perspective of 20 Years Hemodialysis Registry in Iran, on the Road to Progress. IJKD 2020; 14: 95-101.
6. Haseltine WA. Regenerative Medicine 2003: An Overview. J Regen Med 2003; 4: 15-18.
7. Subramanian B, Rudym D, Cannizaro C, Perrone R, Zhou J, Kaplan DL. Tissue-engineered Three-dimensional in vitro Models for Normal and Diseased Kidney. Tissue Eng Part A 2020; 16(9): 2821-2831.
8. Nichols JE, Niles JA, Cortiella J. Engineering Complex Synthetic Organs. In: Bernstein HS (editor) Tissue Engineering in Regenerative Medicine. Humana Press 2011. Springer Science+ Business Media. New York: 299-300.
9. Perin L, Giuliani S, Sedrakyan S, Da Sacco S, De Filippo R. Stem Cell and Regenerative Science Application in the Development of Bioengineering of Renal Tissue. Pediatr Res 2008; 63: 467-471.
10. Rosines E, Johnkura K, Zhang X, Schmidt HJ, DeCambre M, Bush KT, Nigam SK. Constructing Kidney-like Tissues

from Cells Based on Programs for Organ Development: Towards a Method of in vitro Tissue Engineering of the Kidney. Tissue Eng Part A 2010; 16(8): 2441-2455.

11. Fissel WH, Roy S. The Implantable Artificial Kidney. Semin Dialysis 2009; 22(6): 665-670.
12. Nakayama KH, Batchelder CA, Lee CI, Tarantal AF. Decellularized Rhesus Monkey Kidney as Three-Dimensional Scaffold for Renal Tissue Engineering. Tissue Eng Part A 2010; 16(7): 2207-2216.
13. Porzionato A, Stocco E, Barbon S, Grandi F, Macchi V, DE Caro R. Tissue-engineered Grafts from Human Decellularized Extracellular Matrices: A Systematic Review and Future Perspective. Int J Mol Sci 2018; 19: 4117.
14. Stewart DE, Garcia VC, Rosendale JD, Klassen DK, Carrico BJ. Diagnosing the Decades-long Rise in the Deceased Donor Kidney Discard Rate in the United States. Transplantation 2017; 101: 575-587.
15. International Registry in Organ Donation and Transplantation. Available from: irodat.org
16. Orlando G, Booth C, Wang Z, Totonelli G, Ross CL, Moran E, Salvatori M, Maghsoudlou P, Turmaine M, Delario G, et al. Discarded Human Kidney as a Source of ECM Scaffold for Kidney Regeneration Technologies. Biomaterials 2013; 34: 5915-5925.
17. Ross CL, Booth C, Sanders B, Bergman C, Soker T, Stratta RJ, et al. Abdominal Organ Bioengineering: Current Status and Future Perspectives. Expert Opin Biol Ther 2013; 13: 103-113.
18. Peloso A, Petrosyan A, Da Sacco S, Booth C, Zambon JP, O'Brien T, Aardema C, Robertson J, Perin L, Orlando G. Renal Extracellular Matrix Scaffolds from Discarded Kidneys Maintain Glomerular Morphometry and Vascular Resilience and Retains Critical Growth Factors. Transplantation 2015; 99(9): 1807-1816.
19. Moon KH, KO IK, Yoo JJ, Atala A. Kidney Diseases and Tissue Engineering. Methods 2016; 99: 112-119.
20. Bombelli S, Meregalli C, Scalia C, Bovo G, Torsello B, De Marco S, Cadamuro M, Vigano P, Strada G, Cattoretti G, et al. Nephrosphere-derived Cells are Induced to Multilineage Differentiation when Cultured on Human Decellularized Kidney Scaffolds. Am J Pathol 2018; 188: 184-195.
21. Figliuzzi M, Bonandrini B, Remuzzi A. Decellularized Kidney Matrix as Functional Material for Whole Organ Tissue Engineering. J Appl Biomater Funct Mater 2017;15(4):e326-e333.
22. Yang H, Atala A, Yoo JJ. Kidney Regenerative Approaches for Translation. World Journal of Urology 2019. doi. 10.1007/s00345-019-02999-X.
23. Nagao RJ, Xu J, Luo P, Xue J, Wang Y, Kotha S, Zeng W, Fu X, Himmelfarb J, Zheng Y. Decellularized Human Kidney Cortex Hydrogels Enhance Kidney Microvascular Endothelial Cell Maturation and Quiescence. Tissue Eng Part A 2016; 22: 1140-1150.
24. Hiraki HL, Nagao RJ, Himmelfarb J, Zheng Y. Fabricating a Kidney Cortex Extracellular Matrix-Derived Hydrogel. J Vis Exp 2018; 140: 58314.
25. Ross EA, Williams MJ, Hamazaki T, et al. Embryonic Stem Cells Proliferate and Differentiate when Seeded into Kidney Scaffolds. J Am Soc Nephrol. 2009;20 (11): 2338-2347.
26. Bonandrini B, Figliuzzi M, Papadimou E, et al. Recellularization of Well-preserved Acellular Kidney Scaffold Using Embryonic Stem Cells. Tissue Eng Part A 2014;20(9-10): 1486-1498.
27. Remuzzi A, Figliuzzi M, Bonandrini B, et al. Experimental Evaluation of Kidney Regeneration by Organ Scaffold Recellularization. Sci Rep 2017;7: 43502.
28. Abolbashari M, Agcaoili SM, Lee M-K, et al. Repopulation of Porcine Kidney Scaffold Using Porcine Primary Renal Cells. Acta Biomater 2016; 29: 52-61.
29. Song JJ, Guyette J, Gilpin S, Gonzalez G, Vacanti JP, Otto HC. Regenerative and Experimental Orthotopic Transplantation of a Bioengineered Kidney. Nat Med 2013;19(5): 646-651.
30. Montserrat N, Garreta E, Izpisua Belmonte JC. Regenerative Strategies for Kidney Engineering. The FEBS Journal 2016; 283: 3304-3324.
31. Hillebrandt KH, Everwien H, Haep N, Keshi E, Pratschke J, Sauer IM. Strategies Based on Organ Decellularization and Recellularization. Transplant International 2019; 32: 571-585.

Corresponding to:
Alireza Heidary Rouchi, MD, PhD
Liver Transplantation Research Center, Imam Khomeini Hospital Complex, Keshavarz Blvd., Tehran University of Medical Sciences, Tehran 1419733141, Iran
Tel: 0098 21 6119 2659
Fax: 0098 21 6658 1657
E-Mail: rouchiah@sina.tums.ac.ir

Received December 2020
Revised February 2021
Accepted March 2021