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THE BIOENGINEERED KIDNEY – SCIENCE OR SCIENCE FICTION?

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Abstract

Purpose of review—The purpose of this review is to give an overview of important new advances relating to kidney bioengineering.

Recent findings—Directed differentiation studies have shown that proximal tubules, distal tubules, podocytes, collecting ducts, interstitium and endothelial cells can be generated from patient-derived stem cells using standardized protocols. One caveat to the interpretation of these studies is that the physiological characteristics of differentiated cells remain to be defined. Another important area of progress is scaffolding. Both decellularized organs and polymeric materials are being used as platforms for three dimensional growth of kidney tissue and key distinctions between these approaches are discussed.

Summary—In the past 3 years it has become clear that building kidney tissue is feasible. The laboratory-grown kidney is an attainable goal if efforts are focused on refining directed differentiation procedures to optimize cell function and on developing scaffolding strategies that ensure physiological function at the tissue level.

Keywords

Directed differentiation; organogenesis; laboratory-grown tissue

Introduction

Nearly 100,000 Americans are currently waiting for a kidney transplant, but only approximately 17,000 of them will receive organs this year. Research aiming to solve organ shortage has been a priority for many years but progress has been limited. In this piece we aim to review some recent, innovative approaches that may provide practical solutions within the foreseeable future.

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Conflicts of interest

The authors have no conflicts of interest.

Sources of cells for kidney bioengineering

The idea of bioengineering replacement organs has been around for decades but scientific challenges have made this goal seem remote. Although the field of regenerative medicine has seen impressive advances in the differentiation of specific cell types such as the insulin-producing pancreatic beta cell, the idea of engineering a structurally complex organ like the kidney has been viewed with great skepticism. A primary obstacle is the coordinated differentiation and integration of the over 20 distinct cell types found in this organ. Experience indicates that cells isolated from functioning adult kidneys lose their differentiated features once they are cultured *in vitro*. For example, podocytes in culture display limited capacity to form foot processes, and cultured proximal tubule cells do not have brush borders comparable to their counterparts in the kidney. It is unclear if these regressive changes in differentiation state occur because terminally differentiated cells are forced into a proliferative state in culture, or if they simply lack the appropriate environmental cues. Whatever the cause, observations on the behavior of cultured primary cells have made it clear that new approaches to the derivation and culture of functional cell types need to be employed in any serious endeavor to engineer kidney tissue. Recent discoveries have at least partially addressed this deficit.

Several studies have shown that cells grown in 3D culture maintain their *in vivo* phenotype more efficiently than cells grown in monolayer. This principle has been exploited on a miniature scale to develop microfluidic platforms that can be used to model segments of the nephron for functional testing [1]. Although these systems indicate that complex physiological functions can be reproduced in culture, an adequate source of cells has been a limitation for bioengineering on the whole organ scale. How does one generate sufficient quantities of the 20+ cell types present in an adult kidney to piece together a functioning organ? Most of the cells of the adult kidney are derived from 3-4 progenitor cell types present within the intermediate mesoderm and the revolutionary discovery of cellular reprogramming now provides the technology to generate these progenitors in unlimited quantities. Induced pluripotent stem cells (iPSCs) derived from adult donors provide the starting point for the generation of patient-matched replacement tissues using appropriate cellular differentiation protocols [2]. In principle, engraftment of patient-matched tissue would require minimal or no immunosuppression, decreasing side effects which cause significant morbidity and mortality in transplant recipients. The reprogramming procedure is based on transient expression of the KLF5, OCT4, MYC, and SOX2 (KSOM) transcription factors that control the undifferentiated state of stem cells in the early embryo but are lost in differentiated cells. Initial reprogramming efforts involved genetic modification of cells, but refinement of the method has led to the development of non-integrating viral transduction methods for transient expression of the KSOM factors.

Techniques have been developed to sequentially convert iPSCs into intermediate mesoderm and further to differentiated kidney-specific lineages by culture in cocktails of growth factors and signaling pathway inhibitors. Combinatorial testing has led to development of diverse protocols for differentiation of renal lineages, primarily featuring manipulation of the FGF, BMP, activin, WNT, and retinoic acid pathways [3-5]. Ureteric bud cells derived from human iPSC-derived mesoderm integrate in collecting ducts when incorporated in cultured

mouse embryonic kidney rudiments [6]. Similarly, human iPSC-derived nephron progenitor cells are incorporated into nascent nephrons in cultured kidneys [3]. More surprisingly, human iPSC-derived cells can self-aggregate into nascent nephron structures and form epithelial tubules expressing markers of proximal and distal tubules as well as podocytes [7,8]. Perhaps most remarkably, human iPSCs can be differentiated to diverse mixtures of many of the cell types required to form an embryonic kidney, including endothelial cells, interstitial cells, collecting duct cells, cells of the tubular nephron, and podocytes [9]. These distinct cell types self-organize within organoids and can form large clusters containing approximately 500 nephrons, many of which are connected to collecting ducts.

Ongoing studies of self-renewal and differentiation properties of naturally occurring embryonic nephron progenitor cells are providing an understanding of the complex signaling interactions that govern the balance between self-renewal and differentiation of progenitor cells in the developing kidney, and will provide guidance for the refinement of directed differentiation procedures for human iPSCs [10]. Despite efforts to optimize and standardize procedures for stem cell reprogramming and culture, there remain significant differences in differentiation properties between cells reprogrammed from the same individual, presumably due to residual epigenetic modifications that are not fully reversed in the reprogramming process. Analysis of cell-type specific gene regulatory networks can be used to determine the differentiation state of cells. Computational platforms such as Cellnet are extremely powerful as they group cells based on similarity of global gene expression patterns [11]. These platforms will increase in predictive power as more data is accumulated, and it is possible that selection of appropriate iPSC lines for kidney cell differentiation experiments could eventually be entirely computational. However, at present a vital component of studies aiming to differentiate kidney cells from iPSCs is empirical comparison of the efficiency of distinct cell lines.

In summary, morphological and molecular marker analyses indicate that a large number of the cell types essential for a bioengineered kidney can now be generated from directed differentiation of iPSCs in the laboratory, including the podocyte, proximal and distal tubule cells, as well as cells of the collecting duct. However, it seems appropriate at this early point in the development of this technology to emphasize the need for rigorous phenotypic characterization of the cells present in these systems. Although marker analysis can give some confidence in cell phenotype, this technique alone is insufficient and physiological testing is essential. In addition to single-nephron physiology approaches such as microperfusion and transepithelial ion measurements, novel 3D culture methods using microfluidic devices in which single nephron segments are established should be used to study essential functions such as the formation of the glomerular basement membrane [12]. Subjecting laboratory-grown tissues to a battery of functional tests is essential if we are to be confident that we have truly engineered a nephron. Given the accelerating pace of research in the directed differentiation field it seems realistic to believe that cultures will soon be sufficiently robust for such functional testing.

Engineering approaches to kidney structures

While self-organization of tubules in aggregated nephron progenitor cells cultured under differentiation conditions provides proof that cells can form epithelia with lumens, scaling this up to structures containing many thousands of nephrons presents obstacles related to tissue perfusion that need to be solved with 3D biological scaffolding. One approach that has received a lot of attention is reseeded of decellularized organs. Large numbers of kidneys harvested for transplantation are not used because they do not fulfill quality control criteria, but in theory they could still be used as scaffolds for healthy cells. Pigs are another potential source of organs for decellularization as their kidneys have a similar size, shape and physiology to our own. In these protocols, the whole organ is subjected to a series of perfusion washes with detergents and other chemicals that degrade cellular components, resulting in tissue with intact extracellular matrix but lacking cells, that is presumably non-immunogenic. Cells are seeded into the decellularized organ, settle on the remnant extracellular matrix and colonize the cell-free structure. It is unclear if cells home to basement membranes appropriate for their identity or attach randomly to basement membranes that subsequently may influence their differentiation. Access to the vascular network is accomplished through cannulation, but seeding cells into collecting ducts and nephrons is more complex. In one strategy, cells are injected into the vascular network and colonize the nephron, indicating a breach of the blood:urine barrier, perhaps at the glomerular basement membrane [13]. In another approach cells are injected into the renal pelvis and negative pressure is applied to the organ, filling the collecting duct system with cells that presumably reach nephrons through retrograde transport [14]. This intriguing technology is under active development and several questions remain to be answered. For example, it is unclear if the ECM of these scaffolds is altered by the chemical process of decellularization, perhaps inhibiting the development of healthy parenchyma. Kidney recellularization has been reported using newborn rat kidney cell mixtures, primary proximal tubule cells, and primary endothelial cells, and the obvious next challenge is recellularization with cells derived from human iPSCs.

A fundamentally different approach to scaffolding is the use of polymers to engineer structures into which cells can be seeded. One versatile material of particular interest is silk, which has been widely used in surgical applications for centuries and is known not to trigger an immune response [15]. Purified silk can be processed into a large number of formats, including threads, sponges, hydrogels, clear films, and tubular structures. ECM proteins can be incorporated into the silk during the processing, and the surfaces of scaffolds may also be chemically modified, facilitating the development of specific extracellular environments for cell growth [16]. Thus, a single silk scaffold may be regionalized, providing zones with distinct ECM compositions optimal for the attachment, growth and phenotypic maintenance of different cell types. Compared to the whole organ recellularization approach, building tissue from polymeric materials like silk provides the advantage that cells may be systematically arranged as the tissue is assembled. On the other hand, the decellularized organ provides the most accurate representation of the extracellular matrix environment. One interesting distinction that may inform the choice of scaffolding strategy is the source of the cells used for seeding. If naïve progenitor cells are to be used and directed to undergo

differentiation into epithelia, interstitial cells, and blood vessels using developmental pathways, then providing these cells with the extracellular environment of differentiated adult cells may not be optimal. ECM components laid down by cells during development are often transitory, being replaced with mature matrix when the cell achieves its differentiated state [17]. It may therefore be simpler to emulate the embryonic environment for naïve progenitor cells using a scaffolding approach in which the cells lay down and degrade extracellular matrix components specific for their state of differentiation. For experiments in which fully differentiated cells are used for recellularization, there is no doubt that the decellularized adult organ provides the most appropriate extracellular environment.

Obstacles to successful engraftment and function of the bioengineered kidney

Because the science of regenerative medicine for the kidney is at a very early stage, many of the technical problems regarding how to implant laboratory-grown tissue and ensure its vascular integration are unexplored. Innovative solutions to these practical problems are predicted to go hand in hand with the development of ever more complex laboratory-grown kidney tissues. One key area about which we know very little is the immunological profile of differentiating iPSCs. Early in the development of iPSC technology it was hoped that transplantation of autologous reprogrammed cells would sidestep immune tolerance problems. Reports have however contested this, showing that reprogrammed cells do evoke an immune reaction when engrafted into an autologous recipient [18]. A consensus view of why this occurs, and how severe this problem really is remains to emerge from the iPSC field, but a recent report indicates that cells derived from the same iPSCs but of distinct differentiation fates display different immunogenicities [19]. Another important area about which we know very little is the role of age in success of engraftment of iPSC-derived tissue. Studies of injury response in aging mice have revealed that cellular proliferation and repair are reduced with age [20,21]. Laboratory-grown tissue strategies that rely on proliferation and differentiation of graft cells following transplantation may therefore only be appropriate for a certain age bracket. Currently, most experiments are carried out using relatively young rodents, but engraftment studies should ideally be carried out using animals of various ages. Understanding the influence of age on both source tissue and recipient is important yet practically challenging and it is hoped that the regional centers for aging research established by the National Institute of Aging can provide an accessible source of experimental animals for these studies.

Conclusion: laboratory-grown kidneys are an attainable goal

With this opinion piece we have tried to give a balanced view of some of the highly innovative research towards generating new kidney tissue, and also some of the obstacles. Recent years' research has demonstrated that it is possible to generate kidney tissue on a very small scale. But we are a long way from being able to address the needs for organ replacement. Is it feasible to think of engineering tissues for replacement? First, it is important to note that from a therapeutic standpoint, a great deal of good could come from rather incremental advances. In the early stages, it is perhaps reasonable to focus on specific

cell types of central importance. For example, the proximal tubule cell is required to retain sodium, potassium and phosphate as well as resorb water and inactivate a number of drugs. Engineered proximal tubule cells alone may be sufficient for the enhanced dialysis function provided by renal assist devices, which augments traditional dialysis with biological processing of the dialysate and the blood [22]. But the ultimate goal is to engineer an organ composed of all of the cell types in a mature kidney. Is this attainable? We believe it is. With the building blocks currently in hand, we are highly optimistic that rapid advances towards this goal can be made by concerted and collaborative research efforts between cell/developmental biologists, bioengineers and physiologists and focused funding.

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Key points

- There is a severe shortage of transplant organs and therefore a strong incentive to develop bioengineering approaches for the kidney
- Procedures have been developed to differentiate stem cells derived from adult patients to presumptive podocyte, proximal tubule, distal tubule, collecting duct and vascular endothelial cells
- Although kidney cells differentiated from stem cells express appropriate molecular markers, their functional characteristics remain to be determined
- Scaffolding approaches being applied to the generation of kidney tissue include decellularized kidneys and polymer scaffolds
- Recent years' rapid research advances in differentiation of kidney cell types and scaffolding strategies convince us that laboratory-grown kidneys are an attainable goal