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Current strategies and challenges in engineering a bioartificial kidney

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1. ABSTRACT

Renal replacement therapy was an early pioneer in both extra-corporeal organ replacement and whole organ transplantation. Today, the success of this pioneering work is directly demonstrated in the millions of patients worldwide successfully treated with dialysis and kidney transplantation. However, there remain significant shortcomings to current treatment modalities that limit clinical outcomes and quality of life. To address these problems, researchers have turned to using cell-based therapies for the development of a bioartificial kidney. These approaches aim to recapitulate the numerous functions of the healthy kidney including solute clearance, fluid homeostasis and metabolic and endocrine functions. This review will examine the state-of-the-art in kidney bioengineering by evaluating the various techniques currently being utilized to create a bioartificial kidney. These promising new technologies, however, still need to address key issues that may limit the widespread adoption of cell therapy including cell sourcing, organ scaffolding, and immune response. Additionally, while these new methods have shown success in animal models, it remains to be seen whether these techniques can be successfully adapted for clinical treatment in humans.

2. INTRODUCTION

When Dr. Willem Kolff successfully treated his first uremic patient in 1945, he ushered in a new era for artificial organ development. Nearly seventy years later, it is estimated that over two million patients worldwide undergo dialysis treatment (1), and dialysis remains the only viable, long-term extracorporeal organ replacement therapy. Despite the enormous strides in technology and patient care that dialysis has provided, it still does not provide the same long-term mortality benefit of kidney transplantation (2). Increasingly it is being demonstrated that longer and more frequent dialysis treatment confers improved clinical outcomes

compared to standard thrice-weekly therapy (3-6). However, the vast majority, almost 90%, of patients in the United States still receive thrice weekly in-center hemodialysis. While dialysis provides convective and diffusive clearance of uremic toxins, it lacks the crucial metabolic, endocrine, and homeostatic regulation intrinsic to the native kidney. Therefore, conventional in-center hemodialysis falls far short of complete renal replacement.

Currently, kidney transplantation offers the best option for patients with end stage renal disease (ESRD), but is limited by the scarcity of organs. Worldwide there were approximately 76,000 kidney transplants in 2011, which represents less then 10% of the global need (7). In the United States there are approximately 100,000 patients awaiting a donor kidney (8). The scarcity of organ donation remains the largest limitation to widespread transplantation for the treatment of ESRD in high-income countries. Additionally, while immunosuppression regimens have improved short-term graft survival, 10-year graft survival has not changed significantly. Furthermore, in low-income countries, the cost, lack of infrastructure, infectious diseases, and malnutrition further complicates and limits organ transplantation.

In order to address these issues, researchers worldwide have pursued the development of a bioengineered kidney. The hope has been to address the many shortcoming of current renal replacement therapy by replacing the functions of the native kidney with a bioengineered solution. The bioengineered kidney would provide continuous clearance of solutes and fluid balance without the tether of thrice weekly in center hemodialysis, eliminating the associated complications such as infection, access complications, and diminished quality of life. A fully functional engineered kidney would also improve blood pressure control, bone mineral metabolism, and dietary freedom. Another benefit of cell-based therapies is that they provide the intrinsic metabolic and endocrine activity, eliminating the need for costly injectables such as erythropoietin and active vitamin D. A bioartificial kidney could also solve the organ scarcity problem by acting as a bridge therapy to transplant or even completely replace kidney transplantation all together. To accomplish these goals, a number of different approaches to engineering a kidney have been investigated as a sustainable alternative to dialysis and kidney transplantation. This review will examine the current state-of-the art in kidney bioengineering with innovative technologies that span from successful transplantation into animals to successful clinical trials.

3. GROWING A KIDNEY IN-SITU: *DE NOVO* ORGANOGENESIS

De novo organogenesis transplants renal embryonic tissue, metanephros, for in-situ organ development. The developing kidney is derived from the intermediate mesoderm and cells originate from the metanephric blastema and mesonephric duct. The metanephric blastema differentiates into the adult nephron, while the mesonephric duct eventually gives rise to the collecting ducts, ureter, and renal pelvis (9). These embryonic cells, unlike pluripotent stem cells, are preprogrammed to develop and form the complex architecture of a mature kidney. Additionally, metanephroi that have been transplanted exhibit a reduction in immune response because the renal primordia have yet to develop antigen-presenting cells or major histocompatibility complexes (10).

Hammerman and colleagues have transplanted metanephroi for in-situ kidney organogenesis (10). This multi-step process begins with harvesting the metanephros from an embryo. The embryonic tissue is then placed in media containing growth factors that improve their function (11). The tissue is then implanted into the omentum and matures into kidney cortex and medulla (Figure 1). The transplanted tissue develops normal architecture that is identical to native kidney architecture by light microscopy (12). The transplant also becomes vascularized following implantation into the omentum by the host's arteries. The immune response following allotransplant is minimal and has been demonstrated without immunosuppression (13). The transplanted metanephroi have survived as long as 32 weeks and functionally are able to ultrafilter inulin following ureteroureterostomy. The estimated glomerular filtration rate based on inulin clearance was measured to be 6-11% (10,14). The transplants are also able to produce erythropoietin and 1,25-dihydroxyvitamin D_3 demonstrating the ability to perform many of the metabolic and endocrine activities of a native kidney.

The ability of metanephroi transplant to modestly prolong life was demonstrated by Rogers *et al.* (15). The metanephroi of allogenic day 15 rat embryos were transplanted into the omentum and single nephrectomy was performed at the

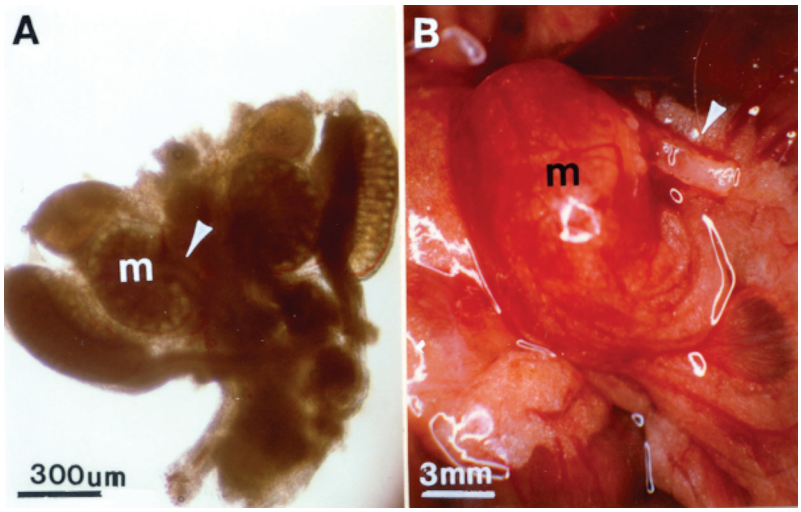

Figure 1. (A) Photograph of retroperitoneal dissection from an E15 rat embryo showing metanephros (m) and ureteric bud (arrowhead). (B) Photograph of a developed metanephros (m) in the omentum of an adult host rat 3 weeks posttransplanation. Arrowhead shows developed ureter. Magnifications are shown (43).

time of implantation. At 3 weeks post-transplant, an ureteroureterostomy was performed on the developing kidney. Then 20 weeks post-transplant, the remaining native kidney was removed from the rats. The survival time was 67 ± 2.7. hours versus 125 ± 12 hours for control and transplanted rats, respectively (15). Rats that underwent transplant, but then had their ureteroureterostomy severed at the time of their second native kidney removal died at the same time as control rats.

Marshall *et al.* demonstrated that survival time was proportional to the amount of metanephroi renal mass that was transplanted (16). Three metanephroi from day 15 rat embryos were transplanted into the peritoneum of recipient rats following unilateral nephrectomy. After 21 days, an ureteroureterostomy was performed. A total of five rats had suitable connections for two-transplanted metanephroi and five had a suitable connection to one transplanted metanephroi. Five weeks after ureteroureterostomy, the remaining native kidney tissue was removed. The animals with no renal mass lived 76.6. ± 9.3. hours. In contrast, the animals with a single connected metanephroi lived for 105.6. ± 13.1 hours and animals with two connected metanephroi lived the longest, 121.2 ± 25.6 hours. The excretory function of transplants was compared to animals that only had unilateral nephrectomy (urine physiology control). The sodium and potassium excretion was significantly lower in the transplants than compared to unilateral nephrectomy controls, but no different between single and double transplants. However, urea excretion concentration was comparable to controls with double transplants, but not in single transplants, indicating that increased renal mass improved urea excretion. *In situ* organogenesis has shown that metanephros transplants can develop into kidney tissue and provide minimal functionality to modestly prolong survival times. It also appears in early studies that transplantation of greater renal mass leads to improved outcomes.

4. SUPPLEMENTING A FAILING KIDNEY: CELL TRANSPLANTATION

Kim *et al.* have transplanted fetal kidney precursors into rats with kidney failure to supplement the native tissue (17). The goal of the technique is for the transplanted precursors cells to develop into healthy kidney cells and augment the function of the failing native kidneys. The use of fetal kidney precursors allows for differentiation into various cell types for the development of complex renal structures. Therefore, using terminally differentiated adult kidney cells may limit complete kidney tissue formation. Another, advantage of using fetal precursors cells is that they are less susceptible to host immune response.

Initially, to mimic the conditions of kidney failure, rats underwent 5/6 nephrectomy and were observed for 5 weeks prior to cell transplantation. Fetal kidney precursor cells were obtained from the

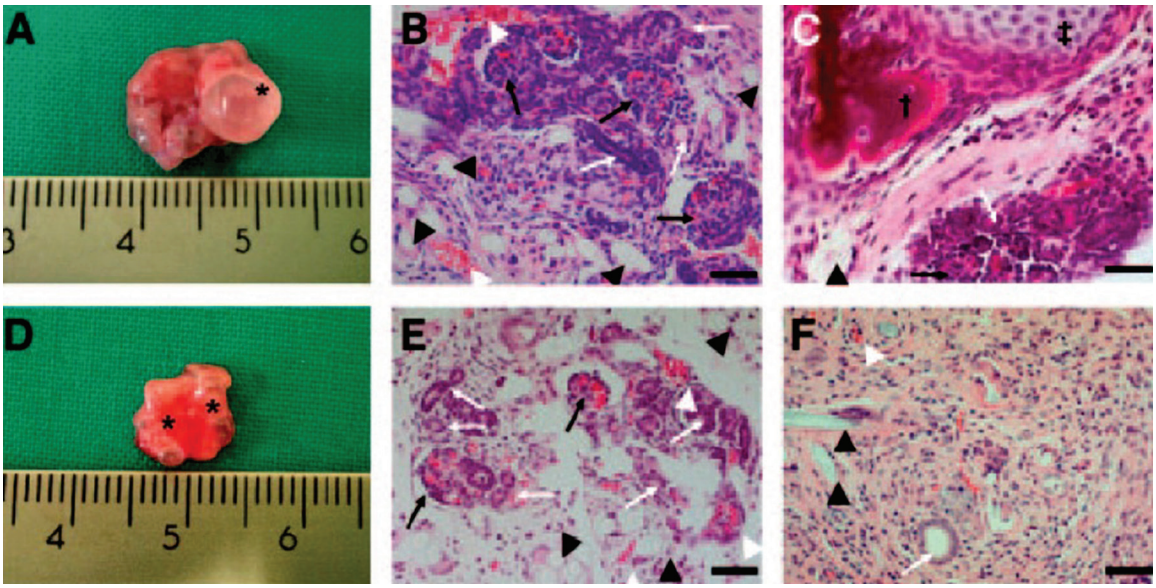

Figure 2. Transplants retrieved from the omentum of immunodeficient mice 3 weeks after transplantation. (A,D): Macroscopic views of (A) E14.5 and (D) E17.5 transplants. Scales in centimeters. The transplants contained large cysts (*). (B): Histological analysis (H&E staining) identified primitive tubules (white arrow), glomeruli (black arrow), newly formed blood vessels (white arrowhead), and polyglycolic acid fibers (black arrowhead) in the E14.5 transplants. (C): E14.5 cells partially differentiated into bone (†) and cartilage (‡). (E): The retrieved E17.5 transplants showed the formation of tubules and glomeruli, (F) but the E20.5 transplants showed very little kidney tissue formation (scale bars = 20 µm) (18).

metanephroi of embryonic day 17.5 rat fetuses and implanted under the remaining kidney capsule of the 5/6 nephrectomized rats. A fibrin gel matrix was used as a three-dimension scaffold and contained 1.2. x 10^8 cells, which were injected into the subcapsular region. The transplants were retrieved at 6 (n = 10) and 10 weeks (n = 10) for analysis. Transplants containing adult kidney cells (n = 20) and those with no cells (n = 20) served as controls. Histological imaging showed the reconstitution of both glomerular and tubular structures with fetal kidney precursor transplants, while no new tissue was seen with the adult cell transplants. Additionally, there was greater evidence of global and focal segmental glomerulosclerosis and tubular atrophy in the control transplants (adult cell and no cell transplants) compared to the fetal cell transplants. Analysis of the animals showed lower serum blood urea nitrogen levels, less proteinuria, and greater creatinine clearance in the fetal cell transplants compared to adult cell transplants and those with no cells. Survival was also enhanced in the fetal cell transplant group with no deaths occurring in fetal cell transplants, while six rats died in the adult cell transplant group and five rats died in the no cell group. There was also less immune reaction in the fetal cell transplants compared to adult cell transplants based on CD4 and CD8 immunohistochemistry staining and mRNA expression.

Kim *et al.* also described the transplantation of fetal kidney cells at different gestational stages in the omentum or injected into the native kidney of immunodeficient mice (18). They isolated fetal kidney cells from rat fetuses at embryonic day 14.5, 17.5, and 20.5 as well as adult kidney cells. A porous polygylcolic acid mesh was used as a three-dimensional scaffold for the omentum transplants and a fibrin gel matrix was injected into the subcapsular and cortex region of the kidney in immunodeficient mice. Three weeks after transplantation, gross examination revealed the formation of cystic fluid (Figure 2). The embryonic day 14.5 cells showed early glomeruli and tubules, but also differentiated into non-renal tissues such as bone and cartilage. The day 17.5 and 20.5, however, did not differentiate into non-renal tissues, but there were fewer nephron structures in the day 20.5 transplants. The fetal cells transplanted in the kidney showed similar results to the omentum transplants. These results show that too early a gestational age (embryonic day 14.5) results in some differentiation into non-renal tissue and too late (embryonic day 20.5) results in diminished regenerated kidney tissue. Therefore,

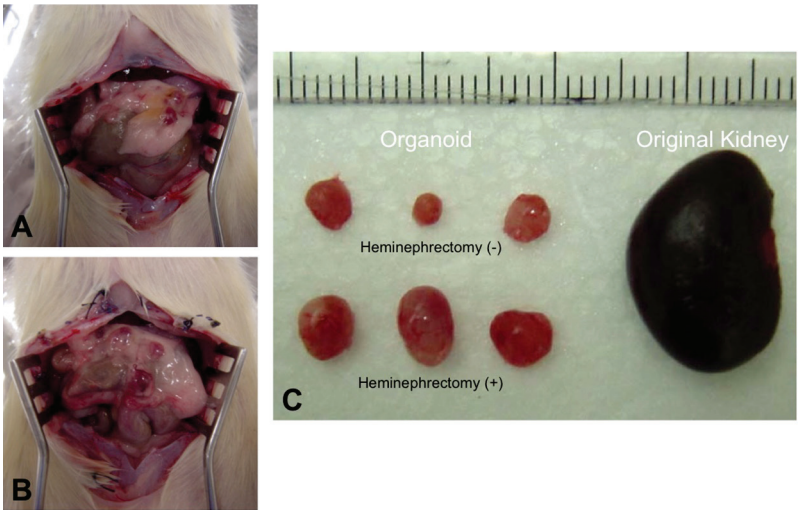

Figure 3. Development of neokidney derived from human mesenchymal stem cells (hMSCs). The neo-kidney was implanted into the omentum of rats without heminephrectomies (A) and with heminephrectomies (B). After this relay culturing, hMSCs formed into an organoid (C). Images courtesy of Dr. Takashi Yokoo, Jikei University School of Medicine.

the timing of gestational age is critical for the optimal development of a functioning kidney.

5. GROWING A KIDNEY IN-VITRO

5.1. Kidney factory: Stem cells

Yokoo *et al.* have developed an *in vitro* organ factory to create kidney tissue from human mesenchymal stem cells (19). The in-vitro factory allows for the production of complex organ structures from autologous adult stem cells by exposing them to the appropriate developmental milieu within an embryo. The technique injects mesenchymal stem cells into the nephrogenic region, and thus provides the appropriate signaling and environment for nephrogenesis. The vascularization and incorporation of the kidney tissue occurs following transplantation into the omentum of the host.

To demonstrate this concept, rat embryos from embryonic day 11.5 were harvested and grown in culture. Bone marrow derived human mesenchymal stem cells were labeled with LacZ using a retrovirus to help distinguish donor-derived cells. The mesenchymal stem cells were also transfected with glial cell line-derived neurotrophic factor to enhance development. The human stem cells were then injected into the nephrogenic region of the rat embryo. The injected embryos were cultured for 48 hours, and then, the metanephroi were dissected out and cultured for an additional 24 hours (20). The developing neo-kidney was implanted into the omentum of rats with and without heminephrectomies and examined after 2 weeks. The neo-kidney (Figure 3) grew within the omentum to 64 ± 21 mg. The mesenchymal stem cells had differentiated into mature structures within the neo-kidney by microscopy and expressed podocyte, tubular epithelial, and endothelial specific genes. The neo-kidneys were also shown to produce human erythropoietin that was responsive to anemia (21). The tissue also became vascularized via several vessels from the omentum and most of the peritubular capillaries were LacZ positive, indicating they were derived from the transplanted mesenchymal stem cells. To assess urine production, the neo-kidney was left in the omentum for a total of 4 weeks and developed hydronephrosis. The fluid was collected from the dilated collecting system and showed elevated levels of urea and creatinine compared to serum. These findings indicate that the neo-kidney was able to produce urine like fluid.

5.2. Cloning a kidney: Nuclear transplantation

Nuclear transplantation has been used by Lanza *et al.* to create cloned renal tissue on polycarbonate membranes derived from adult bovine fibroblasts (22). The adult bovine cell line was created from dermal fibroblasts of adult cows. The nucleus of the adult bovine fibroblasts was transplanted into enucleated bovine oocytes. Renal cells were then

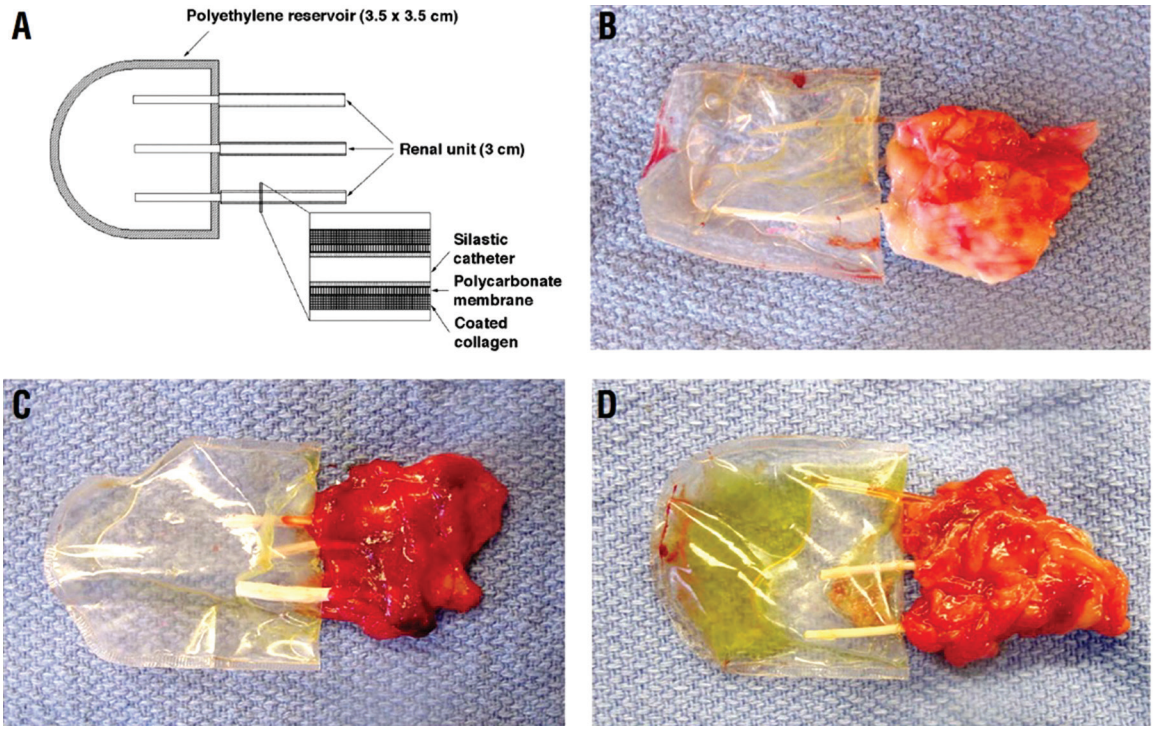

Figure 4. Tissue-engineered renal units. (A) Illustration of renal unit and units retrieved three months after implantation. (B) Unseeded control. (C) Seeded with allogeneic control cells. (D) Seeded with cloned cells, showing the accumulation of urine like fluid (22).

isolated from 56-day old cloned metanephros and expanded. The cells produced renal specific proteins, including aquaporin and Tamm-Horsfall protein as well as 1-25-dihydroxyvitamin D_3 and erythropoietin. The cloned cells were then seeded onto cylindrical polycarbonate membranes that were coated with collagen. The three membranes terminated into silastic catheters connected to a reservoir for urine collection (Figure 4). A total of 31 constructs (n = 19 cloned cells, n = 6 allogenic cells, n = 6 no cells) were implanted subcutaneously for 12 weeks. The cloned constructs were implanted into the same steer that the cells were derived from. Upon retrieval of the constructs, there was vascularization and assembly into glomerular and tubule-like structures. The cloned cell constructs also produced six times more urine then allogenic cell constructs and unseeded constructs. Analysis of the fluid showed greater urea, 18.3. ± 1.8 mg/dl in cloned constructs compared to 5.6 ± 0.3 mg/dl and 5.0 ± 0.1 mg/dl, in allogenic and unseeded constructs, respectively. The same was true for creatinine, 2.5 ± 0.18 mg/dl vs. 0.4 ± 0.18 mg/dl vs. 0.4 ± 0.08 mg/dl, cloned vs. allogenic vs. unseeded, respectively. The cloned constructs were tested for immune reaction via delayed-type hypersensitivity in-vivo and Elispot in-vitro showing no rejection response in the cloned cells.

6. RESEEDING A KIDNEY: DECELLULARIZED SCAFFOLDS

The decellularization process creates an organ scaffold that utilizes the intrinsic extra-cellular matrix and maintains the organ's original architecture. The native cells are first removed using detergents leaving behind only the extra-cellular matrix. The scaffold is then repopulated with cells creating the reseeded organ. Ross *et al.* utilized the extra-cellular matrix signaling to differentiate pluripotent stem cells in rat kidneys (23). Rat kidneys were harvested and decellularized using detergents and displayed both intact microstructure by scanning electron microscopy and contiguous network of collagen IV and laminin via immunohistochemistry. Pluripotent murine embryonic stem cells were perfused either through the arterial or ureteral cannulae. Fluorescence microscopy and light microscopy showed embryonic stem cells populating the glomerular, tubular, and vascular regions. The differentiation of pluripotent embryonic

stem cells was shown by increasing expression of developmental gene, Pax-2 and Ksp-cadherin, observed in later developing kidney cells. The gene expression of these markers were significantly increased multiple fold by day 10 in culture. These observations indicate that matrix signaling may allow for selective differentiation of embryonic stem cells.

Nakayama *et al.* described a similar method to decellularize fetal, infantile, juvenile and adult rhesus monkey kidneys (24). Light microscopy showed the removal of cells and immunohistochemistry confirmed the preservation of extra-cellular matrix proteins heparan sulfateproteoglycan, fibronectin, collagen type I and IV, and laminin. Explants from fetal kidneys were laid on age-matched unrelated donor scaffolds and cultured for 5 days. Cells migrated to the explant-scaffold border, but were not seen within the bulk of the decellularized scaffold. Immunohistochemistry showed cell migration and attachment into the scaffold for reseeding. These studies showed that three-dimensional decellularized scaffolds could provide the signaling and attachment for repopulation.

Recently, Song *et al.* showed that reseeded decellularized kidney scaffold produce dilute urine after orthotropic implantation into rats (25). They had previously reported a similar strategy to bioengineer a heart (26) and lung (27). Using detergents, SDS and Triton X-100, they were able to remove the majority of cells from a cadaveric rat kidney, while still retaining the extra-cellular matrix architecture. The total DNA content was less then 10% compared to native kidneys, but collagen content was unchanged. The decellularized scaffold was repopulated with endothelial cells (human umbilical vein cells) via perfusion through the renal artery. The vascular channels throughout the kidney were lined with endothelial cells after 3-5 days in culture. The epithelial cells were then repopulated by infusing cells through the ureter using neonatal kidney cells. These cells were isolated from renal tissue slurry and consisted of a variety of epithelial phenotypes (8% glomerular phenotype, 69% proximal tubule phenotype and 25% distal tubule phenotype). The constructs were cultured for up to 12 days (Figure 5), but repopulation of the scaffold was observed as early as 4 days. Histologic evaluation showed site-specific engraftment with cells populating extra-cellular matrix regions based on their phenotype. Immunostaining showed that podocytes preferentially seeded to glomeruli and tubular cells organized in tubular structures. *In vitro* transport studies showed that the reseeded kidney had a creatinine clearance of 10% compared to cadaveric kidneys. The reseeded kidneys were also able to retain albumin and reabsorb glucose better then a decellularized scaffold alone, but not nearly as effectively as a cadaveric kidney. These differences were attributed to the relative immaturity of cells and seeding efficiency; the reseeded kidney had 70% of the number of glomeruli compared to cadaveric kidneys.

Orthotropic transplantation of the reseeded kidney in singly nephrectomized rats showed blood flow through the corresponding vasculature and urine production. The rats underwent left nephrectomy and a reseeded left kidney was transplanted. There was no evidence of bleeding or thrombi within the vascular system or evidence of hematuria. The transplanted kidney produced less urine than the native control, 1.2 ± 0.1 µl min^{-1} vs. 3.2 ± 0.9 µl min^{-1}, respectively. Additionally, there was less creatinine clearance and urea excretion compared to native controls. The in-vivo studies mirrored the *in vitro* studies showing the relative immaturity of cellular constructs.

The proof of concept studies using decellularized scaffolds shows the feasibility of such an approach. The authors also used the same protocol to decellularize porcine and human kidneys to demonstrate that the process could be scaled up to clinically relevant sizes. The reseeded kidney in rats was able to produce urine and showed rudimentary transport of solutes. However, further work is continuing to improve the cell seeding efficiency, up scaling of organ culture, and improvements in transport characteristics in order to achieve clinical viability.

7. BIOENGINEERING A KIDNEY

7.1. Renal assist device (RAD)

Current renal replacement therapy relies on hollow-fiber polymer membranes to provide the diffusive and convective clearance needed for the treatment of ESRD. The addition of cell therapy techniques in conjunction with hollow-fiber membranes aims to provide the metabolic, endocrine, and immunomodulatory functions that current renal replacement therapy lacks. To this end, Humes and colleagues developed the Renal Assist Device (RAD), shown in Figure 6, which consisted of primary renal cells seeded onto hollow-fibers of a standard hemofilter (28). The cells were grown on the inner surface of the hollow-fibers that provided

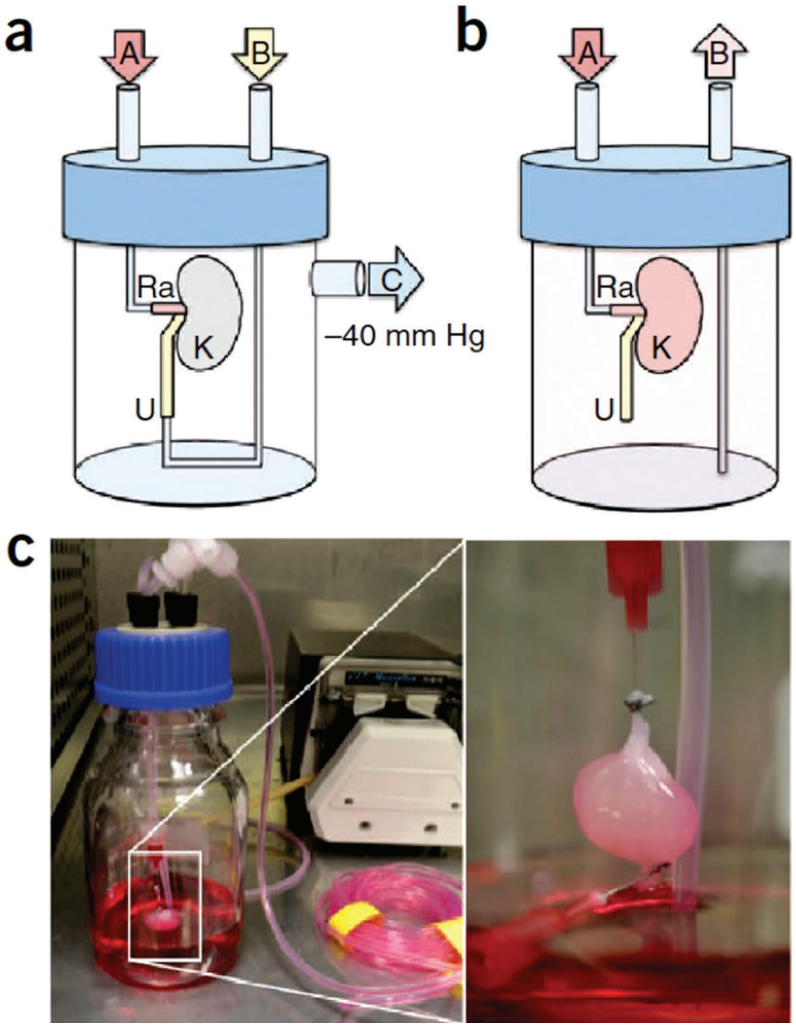

Figure 5. Cell seeding and whole-organ culture of decellularized rat kidneys. (a) Schematic of a cell-seeding apparatus enabling endothelial cell seeding through port A attached to the renal artery (Ra) and epithelial cell seeding through port B attached to the ureter (U) while negative pressure in the organ chamber is applied to port C, thereby generating a transrenal pressure gradient. (b) Schematic of a whole-organ culture in a bioreactor enabling tissue perfusion through port A attached to the renal artery and drainage to a reservoir through port B. K, kidney. (c) Cell-seeded decellularized rat kidney in whole-organ culture (25).

both a mechanical scaffold and immune barrier. The seeded cells also provided active transport as well as metabolic and endocrine activity. In a Phase II randomized, controlled, open-label trial, the RAD was tested in 58 patients with acute kidney injury. The RAD therapy had a remarkable statistically significant mortality benefit at 28 days compared to standard continuous venovenous hemofiltration, 33% vs. 61%, respectively (29). To date, the RAD remains the only viable bioartificial kidney device that has been successfully used in humans. Unfortunately, a follow-up Phase IIb was suspended after interim analysis showed an unexpectedly high survival rate in patients treated with control sham RAD without seeded cells (30). However, this observation has led to additional therapeutic approaches including the bioartificial renal epithelial system, and the implantable renal assist device.

7.2. Bioartificial renal epithelial cell system (BRECS)

The Bioartificial Renal Epithelial Cell System (BRECS) aims to address the important issues of cell cryopreservation, storage, and

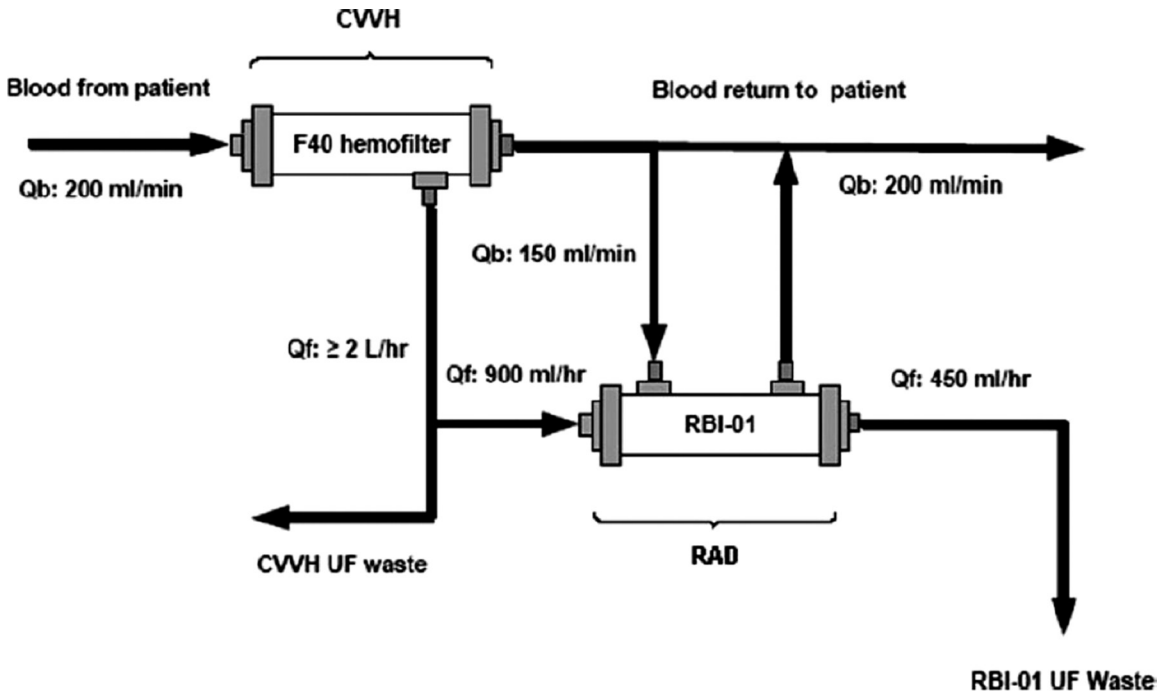

Figure 6. Schematic of the extracorporeal perfusion circuit for renal cell therapy. Flow rates approximate those used clinically. The hemofilter perfusion PUMP system used the BBraun's (Bethlehem, PA) Diapact System; the RAD perfusion system used an Alaris (San Diego, CA) intravenous pump for the pre-RAD ultrafiltrate line and a Minntech (Minneapolis, MN) blood pump for the post-RAD blood line. Qb, blood low; Qf, rate of fluid filtration (29).

distribution for the widespread adoption of renal cell therapy. The BRECS cultures renal epithelial cells onto a cell scaffold made of porous niobium coated carbon disks until there are ~ 10^8 cells (31). The BRECS is then cryopreserved for storage, and upon reconstitution 1 to 3 months later the cells maintain viability, phenotype and metabolic activity. The BRECS also functions as a delivery system and was designed to be used with ultrafiltrated blood or in a peritoneal dialysis setup. The device has shown promising results in extracorporeal large animal studies (30). The BRECS offers an all-in-one system for cell culture, cryostorage and cell therapy delivery system for point of care treatment of kidney injury.

7.3. Implantable renal assist device (iRAD)

The RAD by Humes *et al.* demonstrated that a hemofilter plus cell bioreactor in series recapitulated many of the functions of a native kidney on a macroscale. This has lead to the effort to create a miniaturized version of the RAD that would be suitable for implantation and provide the benefits of a transplanted kidney. Microelectromechanical systems (MEMS) technology has been utilized to tackle these challenges and develop an Implantable Renal Assist Device (32,33) shown in Figure 7. MEMS technology borrows techniques from the semi-conductor and microelectronics industry to create extremely precise and tunable geometries with feature sizes on the nanometer scale, while maintaining a cost effective batch fabrication approach. The use of silicon nanopore membranes (SNM) (Figure 8) has been pioneered for use as both a novel hemofilter as well as an immunoisolatory scaffold for a cellular bioreactor (32-37). The ultrathin SNM have a uniform slit pore design bestowing much higher hydraulic permeability and selectivity compared to the roughly circular-shaped pores in standard hollow-fiber membranes (38). The SNM can have pore sizes as small as 5nm with less than 1% variability (32). Therefore, these membranes function much like the glomerulus of the native kidney by selectively filtering solutes based on molecular weight cut-offs. The selectivity of SNM has been shown in-vitro using various globular proteins (32) as well as $ß_2$-microglobulin (39). The high hydraulic permeability of these membranes allows for a filtration rate of 30ml/min using only the arterial-venous pressure differential, negating the need for an internal pump or external connections.

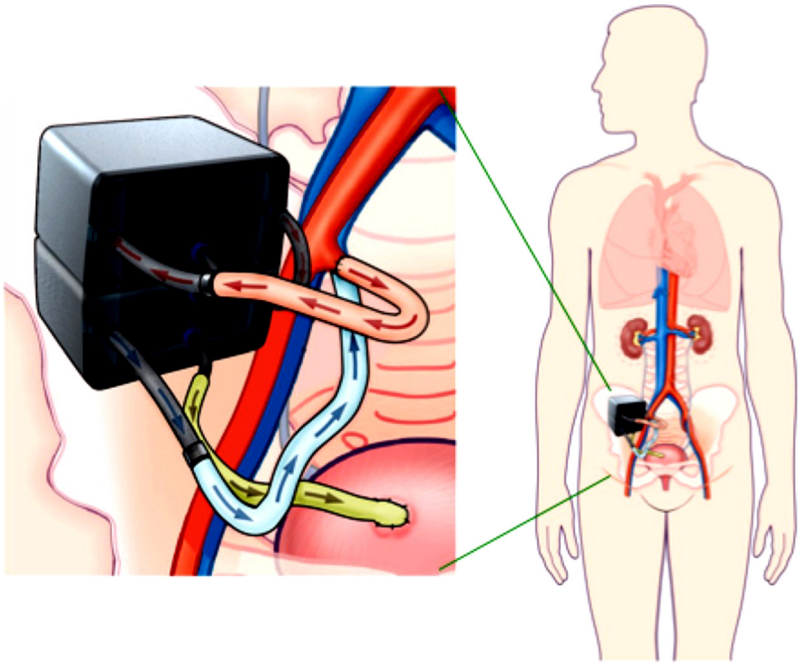

Figure 7. Schematic of the implantable bioartificial kidney. Arterial and venous connections depict blood flow through the device. A conduit connects the device to the bladder for waste removal. The unprecedented hydraulic permeability of the silicon nanopore membranes enables blood to flow through the device with only the arterial venous pressure differential, negating the need for an internal blood pump.

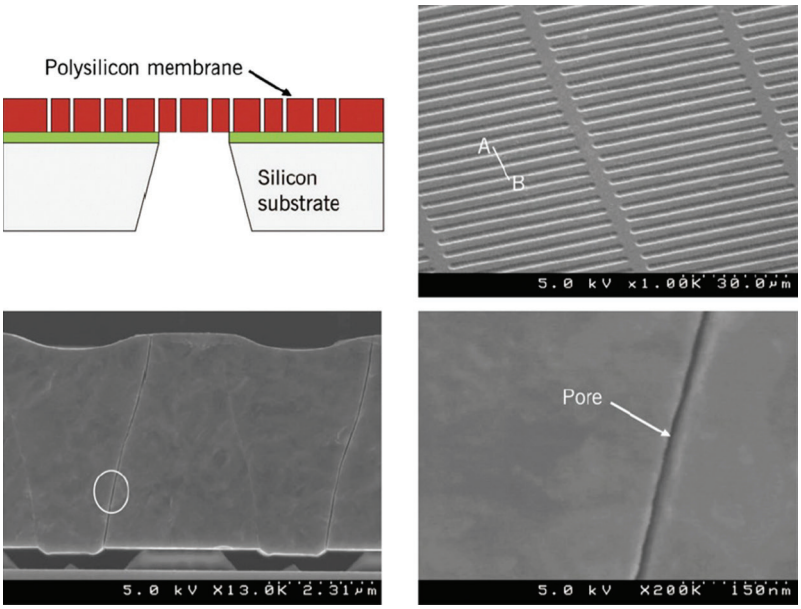

Figure 8. Nanopore membrane fabricated using silicon MEMS technology. *Top left:* Cross-section of membrane illustrating various structural layers (not to scale). Pores (exaggerated) are formed in the polysilicon diaphragm, which is supported by an underlying silicon substrate. *Top right:* SEM image of membrane showing uniformly spaced array of slit pores. *Bottom left:* SEM image showing membrane cross-section and non-tortuous pore geometry. *Bottom right:* SEM image showing close-up of 9nm slight pore and smooth surface characteristics (32).

The long-term stability of hemofiltration was demonstrated for almost 100 hours of continuous in-vitro filtration using anti-coagulated blood. The miniaturization capabilities inherent to MEMS technology will also allow for a total surface area of 0.1.m^2 for the device (32,33).

The second segment of bioartificial kidney acts much like the native tubules by reabsorbing water and solutes and excreting waste while providing an immune barrier. The cellular bioreactor will consist of renal epithelial cells seeded on a SNM scaffold. The membrane will provide both mechanical support for the cells as well as isolate the cells from the host immune system. The cells will also provide the intrinsic metabolic, endocrine, and immunomodulatory functions of the native kidney. The feasibility of this strategy was tested by seeding human cortical epithelial cells to confluence on SNM (40). The cells formed tight junctions, central cilia, and had transepithelial resistances similar to polyester membranes. These studies illustrate the potential for a miniaturized bioartificial kidney and pave the way for continued development.

8. DISCUSSION

Currently, dialysis and kidney transplant remain the only viable option for ESRD patients. However, shortcomings with both treatment modalities limit their long-term effectiveness. Several new innovative approaches hope to bioengineer a kidney and change the current paradigm for renal replacement therapy. These new technologies will have to address their own set of issues including cell sourcing, organ scaffolding, and host immune response.

The use of immature cells, either stem cells or kidney cell pre-cursors, has been the predominate choice for many techniques. This is largely due to their ability to populate the numerous different cell types within the kidney. However, a major challenge of stem cells is to correctly differentiate them into the appropriate phenotype. To address this Yokoo *et al.* have taken advantage of the signals provided by a growing embryo by placing the stem cells in the region of nephrogenesis in embryos. Alternatively, Ross *et al.* has used the signals provided by a decellularized extra-cellular matrix for differentiation into the appropriate kidney cell phenotypes. The use of kidney precursor cell has also shown success, by using metanephroi from embryos to grow organs in-situ or by cell transplantation. Both of these techniques used metanephroi cells from rodent embryos and demonstrated a survival benefit, albeit a limited one. The transition of these techniques to human kidney cells would require human embryos for cell sourcing and clearly raises serious ethical concerns. Aside from the ethical concerns, while all of these techniques have shown varying amounts of success, there still remain major questions regarding large scale cell sourcing to achieve widespread adoption. The culturing of large quantities of progenitor cells remains a challenge. However, Westover *et al.* recently described a novel technique to enhance the propagation of adult human renal epithelial progenitor cells for use in tissue-engineered devices (41). Human kidney tissues were obtained from transplant discards and propagated using an enhanced propagation (EP) protocol that modified the enzymatic digestion, decreased cell plating density, and added retinoic acid compared to standard protocol. This resulted in a significant increase in yield of progenitors cells with more than 10 doublings using the EP technique. This approach would ultimately allow a single 50 g kidney cortex to supply over 5000 tissue engineered therapeutic devices (10^8 cell load per device). Functionally, the cells were also able to maintain their ability to respond to oxidative stress and endotoxin challenge. The cultured cells were then successfully reconstituted after cryopreservation and integrated into renal cell therapy devices. This method represents a robust solution to the cell sourcing problem for cell therapy based devices.

The logistical consideration such as cryopreservation, storage, and distribution is another major hurdle to creating a commercially viable bioartificial kidney. The BRECS, also being developed by Humes *et al.*, aims to address these critical issues by creating a single platform for cell culture, storage and delivery. This technology will further enable the wide spread adoption and commercialization of cell based therapies for kidney disease.

The scaffolds onto which cells are seeded are an important consideration especially for multi-functional organs such as the kidney. The scaffold not only needs to provide mechanical robustness for the organ, but also provide the architecture for cell signaling and cell-cell interactions. The decellularized scaffold most recently demonstrated by Ott and colleagues has shown the feasibility of such an approach and the importance of an intact extra-cellular matrix in cell seeding. The decelluarization

process was scaled to human kidneys and the next logical step would be to demonstrate seeding of those human kidneys. Despite these promising studies, there remains challenges including damage to extra-cellular matrix proteins during decellularization, sterilization techniques, and immune reaction to the extra-cellular matrix (42).

The immune reaction to transplanted tissue has been an ongoing battle since the first attempts at kidney transplantation. New techniques to create bioartificial kidneys will face similar challenges as well as unique immune issues based on the technology used. For example, a major question that remains for decellularized scaffolds is how antigenic epitopes on the extra-cellular matrix will interact with the host's immune system following transplantation. While extra-cellular matrix proteins are highly conserved there will need to be further studies to address these immunologic concerns. The use of kidney precursor cells has been shown to limit the host's immune reaction (17) although it remains unclear if the immune reaction would increase as the cells mature. An alternative method to limiting the immune reaction is immunoisolation by encapsulating cells via a physical barrier. Therefore, SNM produced using MEMS technology achieves tunable pore sizes designed to exclude the passage of antibodies. In this way, the transplanted cells are protected from the host's immune response, but still enables the transport of small molecules (salts, uremic toxins) and water. This unique and versatile method still needs to be validated in long-term in-vivo studies.

To date, the only bioartificial kidney that has shown efficacy in human trials has been the RAD developed by Humes. The RAD demonstrated the proof-of-concept that a biomimetic device could be used to treatment kidney failure. However, the extra-corporeal RAD is impractical for implantable therapy due to characteristics of hollow-fiber dialysis membranes and cumbersome machinery used in the system. Therefore, SNM were developed with unprecedented hydraulic permeability that negates the need for an internal pump and compact geometry that enables an implantable system. Additionally, the BRECS demonstrates that it is possible to overcome many of the logistical obstacles associated with cell therapy. Together, the iRAD concept and advances in the BRECS are advancing towards the goal of making an implantable bioengineered kidney a viable reality.

9. CONCLUSIONS

Renal replacement therapy is an early pioneer in organ replacement. Dialysis, to this day, remains the only long-term extra-corporeal treatment system that is able to provide life-sustaining therapy for a failing organ. This in turn has allowed patients to live for years awaiting a donor kidney on the transplant list. Currently, innovative research is being conducted to address the numerous shortcomings of current renal replacement therapy. These approaches aim to create a fully functional kidney replacement utilizing a cell based therapy approach. Early work has shown promise by developing functional kidney tissue and even prolonging survival following transplantation in animals. Human studies have shown that a cell bioreactor can improve patient's mortality in acute kidney injury. Despite these early successes, there are obstacles to overcome before a bioartificial kidney will become standard of care. A major issue for the field is cell sourcing. There are ethical concerns over using human embryos and fetal cells for organ development. Furthermore, logistical problems regarding cell-sourcing including cell quantity, cryopreservation, storage and distribution remain before wide-spread adoption can occur. There also remain questions regarding scaffold design and architecture. Additionally, transplantation of donor cells will always raise concerns over immune response in the recipient. Finally, the field will need to move beyond rodents and demonstrate the feasibility of their techniques in larger animals and eventually humans. The current landscape for the development of a bioartificial kidney remains robust with promising new technologies on the horizon. However, the development is still nascent with additional work needed to demonstrate viability in human patients.

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