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Cell-Based Approaches for Renal Tissue Regeneration

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ABSTRACT

The kidneys serve a number of important roles that are required to maintain normal human physiologic function. Chronic kidney disease is a leading cause of mortality and morbidity, and a substantial number of these patients progress to end-stage renal disease. End-stage disease involves multiple organ systems and requires renal replacement therapy. Currently, the gold-standard treatment for this condition is renal transplantation, which can restore complete kidney function. However, renal transplantation is limited by the critical shortage of transplant organs and by complications that can result from chronic immunosuppressive therapy and graft failure. Recent advances in cell technologies have allowed for development of cell-based approaches for kidney tissue regeneration. Efforts are ongoing to identify reliable cell sources, develop ideal growth environments and innovative differentiation factors, and discover synthetic and naturally-derived materials for use as an ideal support structure for tissue regeneration. However, numerous challenges must be met in order to translate these techniques into clinically relevant therapies.

INTRODUCTION

The kidneys serve a number of important roles that are required to maintain normal human physiologic function. They are the primary organs for maintaining fluid and electrolyte balance, and they play a large role in maintaining acid-base balance. They produce renin, which is vital to controlling blood pressure, and erythropoietin (EPO), which regulates red blood cell production. They affect calcium metabolism, particularly calcium absorption, by converting a precursor of vitamin D to the most active form of 1,25-dihydroxyvitamin D [1].

When renal tissue is extensively damaged, tubular atrophy and glomerulosclerosis develop; these eventually lead to loss of functional nephron units and fibrosis [2]. Chronic kidney disease (CKD) is a leading cause of mortality and morbidity in Western countries. It affects approximately 11% of the adult population and a substantial number of these patients progress to end-stage renal disease (ESRD), which currently has no cure [3,4]. ESRD is a devastating condition that involves multiple organ systems in affected individuals and requires renal replacement therapy [3,5-10]. Currently, the most common form of renal replacement therapy used

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Abbreviations and Acronyms

3-D = 3-dimensional
ESRD = end-stage renal disease
EPO = erythropoietin
hAFSC = human amniotic fluid-derived stem cell
MSC = mesenchymal stem cell
PEC = parietal epithelial cell
RT-PCR = reverse transcriptase polymerase chain reaction
Sca-1 = stem cell antigen-1

in ESRD is dialysis. However, dialysis neglects the resorptive, homeostatic, metabolic, and endocrinologic functions of the kidney and only partially replaces its filtration properties [11-15]. Renal transplantation is the only definitive treatment that can restore complete kidney function, including filtration, production of EPO, and production of 1,25 dihydroxyvitamin D_3. However, renal transplantation also has limitations, such as a critical shortage of transplant organs, complications due to chronic immunosuppressive therapy, and graft failure [11-13;16-19].

The limitations of current therapies for renal failure have led investigators to explore the development of alternative therapeutic modalities that can improve, restore, or replace renal function. The emergence of cell-based therapies using tissue engineering and regenerative medicine strategies has presented alternative possibilities for the management of pathologic renal conditions [11,20-24]. Kidney cell expansion for cell transplantation using tissue engineering and regenerative medicine techniques has been proposed as a method to augment either isolated or total renal function. Despite the fact that the kidney is one of the more challenging organs to regenerate due to its complexity, investigative advances made to date have been promising [21-23].

FUNDAMENTALS OF RENAL TISSUE REGENERATION

The kidney is a complex organ with multiple cell types and a complex functional anatomy that renders it one of the most difficult to reconstruct [26-32]. The system of nephrons and collecting ducts within the kidney is comprised of multiple functionally and morphologically distinct segments. Thus, techniques in regenerative medicine are directed toward providing appropriate conditions for the long-term survival, differentiation, and growth of many types of cells. Moreover, optimal growth conditions have been extensively investigated to provide adequate enrichment for achieving stable renal cell expansion systems [33-37].

Isolation of particular cell types that produce specific factors, such as EPO, may be a good approach for selective cell therapies. However, total renal function would not be achieved using this approach. To create kidney tissue that would deliver full renal function, a culture containing all of the major cell types comprising the functional nephron units should be used. Optimal culture conditions to nurture renal cells have been extensively studied, and the cells grown under these conditions have been reported to maintain their cellular characteristics. Furthermore, when these cultured renal cells were placed in a 3-dimensional (3-D) culture environment, they were able to reconstitute renal structures [38].

Investigative efforts designed to identify a reliable cell source have also been expanded to include stem and progenitor cells. The use of these cells for tissue regeneration is attractive due to their ability to differentiate and mature into specific cell types that are required for regeneration. This is particularly useful in instances where primary renal cells are unavailable due to extensive tissue damage. Bone marrow-derived human mesenchymal stem cells (MSCs) have been shown to be a potential source, and these cells have the ability to differentiate into several cell lineages [39-41]. These cells have been shown to participate in kidney development when they are placed in a rat embryonic niche that allows for continued exposure to a repertoire of nephrogenic signals [42]. However, it has been shown that bone marrow MSCs contribute mainly to regeneration of damaged glomerular endothelial cells after injury. Instead, the major cell source for kidney regeneration was found to originate in the intrarenal cells in an ischemic renal injury model [39,43]. Recently, the isolation of a side population of cells in the adult human kidney was described. These authors previously showed that side population cells from a mouse kidney can differentiate into multiple lineages [44]. Circulating stem cells have also been shown to transform into tubular and glomerular epithelial cells, podocytes, mesangial cells, and interstitial cells after renal injury [40,45-50]. Another promising stem cell source for renal tissue regeneration is human amniotic fluid-derived stem cells (hAFSCs). These cells are capable of contributing to the development of primordial kidney structures, including the renal vesicle and C-shaped and S-shaped bodies [22]. These observations suggest that controlling stem and progenitor cell differentiation could lead to successful regeneration of kidney tissues.

Although isolated cells have the ability to remodel and tend to form appropriate tissue structures *in vitro*, they can do so only to a limited degree. In addition, tissue particles cannot be implanted *in vivo* in large volumes without a support substance. If they are located more than a few hundred micrometers from the nearest capillary, cells will not survive due to diffusion limitations [51]. Thus, a cell-support matrix is necessary to allow the diffusion of nutrients across short distances. The construct would become vascularized in concert with the expansion of the cell mass [11].

Authors have studied a variety of synthetic and naturally derived materials for the detection of ideal support structures for the regeneration [52-56]. Biodegradable synthetic materials, such as polylactic and polyglycolic acid polymers, have been used to provide structural support for cells. Synthetic materials can be easily fabricated and configured in a controlled manner, which makes them attractive options for tissue engineering. However, naturally derived materials, such as collagen, laminin,

and fibronectin, are more biocompatible and provide cells with an extracellular matrix environment that is similar to normal tissue. For this reason, collagen-based scaffolds have been used increasingly for many applications [57-60].

APPROACHES FOR RENAL TISSUE REGENERATION

Developmental Approaches

Transplantation of kidney precursors such as the metanephros into a diseased kidney has been proposed as a possible method for achieving functional restoration. In a study by Dekel et al [61], human embryonic metanephroi transplanted into the kidneys of an immunodeficient mouse model developed into mature kidneys. The transplanted metanephroi produced urine-like fluid but failed to develop ureters. The results of this study suggest that development of an *in vitro* system in which metanephroi can be grown may lead to transplant techniques that produce a small replacement kidney within the host. On the other hand, Steer et al [62] showed that the metanephros was divided into mesenchymal tissue and ureteral buds, and each of the tissue segments was cultured *in vitro.* After 8 days in culture, each portion of the tissue had grown to the size of the original mesenchymal tissue from which it was derived. A similar method was used for ureteral buds, which also propagated. These results indicate that if the mesenchyme and ureteral buds were placed together and cultured *in vitro*, a metanephros-like structure would develop. The results also suggest that the metanephros could be propagated under optimal conditions.

In a different study, transplantation of metanephroi into the omentum of a nonimmunosuppressed rat was also performed. The implanted metanephroi were able to undergo differentiation and growth that was not confined by a tight organ capsule [63]. When metanephroi with an intact ureteric bud were implanted, the metanephroi enlarged and became kidney-shaped within 3 weeks. In addition, they were able to develop a well-defined cortex and medulla. Mature nephrons and collecting system components taken from these kidney-like structures were shown to be indistinguishable from those of normal kidneys by light and electron microscopy. Moreover, these structures became vascularized via arteries that originated at the superior mesenteric artery of the host [64]. The transplanted metanephroi survived for up to 32 weeks post-implantation [65]. Rosines et al [66] recently devised a method based on the stages of metanephric kidney development. The method was used to engineer *in vitro* kidney-like tissue containing functional tubular transporters and glomeruli with apparent early vascularization. The Wolffian duct was isolated from timed-mated Holtzan rats at embryonic day 13 and induced to bud *in vitro*. Then, each isolated bud was induced to undergo branching. The branched ureteric buds were recombined with metanephric mesenchyme that had been isolated from rat kidney rudiments. After 4 to 6 days of mutual induction, the recombined tissue resembled a late-stage embryonic kidney that was implanted into a host animal. After 14 days, renal structures such as glomeruli and evidence of early vascularization were observed. These studies show that the developmental approach may be a viable option for regenerating renal tissue for functional restoration.

One of the key factors for successful tissue regeneration is controlling kidney differentiation. In another study by Rosines et al [67] hyaluronic acid was shown to possess the ability to simultaneously modulate ureteric bud branching, promote mesenchymal-to-epithelial transformation, and promote differentiation of both metanephric mesenchyme and the ureteric bud depending on its concentration and molecular weight. These findings suggest that hyaluronic acid might be useful for creating a 3-D scaffold for *in vitro* kidney engineering and for promoting tubule regeneration in injured or cryopreserved kidneys, based on the biocompability and crosslinking capability of this compound. Some groups have also described the formation of specific kidney structures *in vitro*. In particular, a new method for creating kidney tubules has been reported. Using micromachined molds, channels were formed in extracellular matrix gels and subsequently filled with Madin-Darbin canine kidney epithelial cells. Upon cell adherence, a second layer of extracellular matrix gel was introduced. After gelation of the second gel layer, the samples were placed into a 12-well plate filled with culture media to allow the cells to assemble into tubular tissue. After 3 to 5 days, the epithelial cells self-assembled into tubular structures of up to 1 cm. These structures developed a lumen lined by a monolayer of polarized epithelial cells at 7 days. Kidney epithelial cells are characterized by their polarization, which is represented by the establishment of specific basolateral and luminal surfaces. The authors conclude that this method is feasible for generating kidney epithelial tubules with natural shape and dimensions in a short time [68].

Interesting methods of creating kidney structures *in vitro* that may optimize *in vivo* growth and differentiation of renal stem cells have been recently studied. Perin et al [22] used nongenetically modified stem cells derived from human amniotic fluid to demonstrate the development and differentiation into *de novo* kidney structures during organogenesis *in vitro*. Human AFSCs were isolated from human male amniotic fluid obtained between 12 and 18 weeks of gestation. Green fluorescent protein and lac-Z-transfected hAFSCs were microinjected into murine embryonic kidneys (12.5-18 days gestation) and were maintained in a special coculture system *in vitro* for 10 days. The hAFSCs were characterized during their integration and

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differentiation in concert with the growing organ. The hAFSCs were capable of contributing to the development of primordial kidney structures including renal vesicle and C-shaped and S-shaped bodies. Reverse transcriptase polymerase chain reaction (RT-PCR) confirmed expression of early kidney markers for zona occludens-1, glial-derived neurotrophic factor, and claudin. The results of this study showed that human amniotic fluid-derived stem cells may represent a potentially limitless source of ethically neutral, unmodified pluripotential cells for kidney regeneration. Similarly, Hu et al [69] mounted embryonic renal tissue containing stem and progenitor cells within a perfusion culture container at the interface of an artificial interstitium made from polyester. The space between the inner wall and the growing tissue was filled with an artificial interstitium made of a polyester fleece to reduce the dead volume space. During the entire culture period, the fleece was in contact with the mounted tissue. In this way, the culture medium flowed through the fleece as it would in natural capillaries and ensured an equally distributed liquid exchange. Thus, the dead space volume was minimized and a constant fluid environment was created within the culture container. Moreover, mechanical protection was provided to the growing tissue. Renal tubules developed in chemically defined Iscove's modified Dulbecco's medium without serum and without coating with extracellular matrix proteins.

Regenerative Medicine Approaches

One of the essential requirements for renal tissue engineering is the ability to grow and expand renal cells. The feasibility of achieving renal cell growth, expansion, and *in vivo* reconstitution using tissue engineering techniques has been investigated [52]. In this study, donor rabbit kidneys were dissociated, culture-expanded, and seeded onto biodegradable polyglycolic acid scaffolds followed by implantation in athymic mice. Histological examination of the implants demonstrated progressive formation and organization of nephron segments within the polymer fibers over time. Renal cell proliferation in the cell-polymer scaffolds was detected by *in vivo* labeling of replicating cells with the thymidine analog bromodeoxyuridine (BrdU). These results established that renal-specific cells can be successfully harvested and cultured, and can subsequently attach to biodegradable substrates. Although initial experiments showed that implanted cell-polymer scaffolds facilitated the formation of renal tubular structures, it was unclear whether the tubular structures reconstituted *de novo* from dispersed renal elements, or if they merely represented fragments of donor tubules that survived the original dissociation and culture processes intact. Fung et al [70] further investigated this topic using mouse renal cells that were harvested and expanded in culture. Subsequently, single isolated cells were seeded on biodegradable polymers and implanted into immune-competent syngeneic hosts. Sequential analyses of the retrieved implants over time demonstrated that renal epithelial cells first organized into a cord-like structure with a solid center. Subsequent canalization into a hollow tube could be seen after 2 weeks. Histological examination with nephron segment-specific lactins showed successful reconstitution of proximal tubules, distal tubules, loops of Henle, collecting tubules, and collecting ducts. These results confirm that single suspended cells are capable of reconstituting into tubular structures, with homogeneous cell types within each tubule.

The kidneys play a major role in body homeostasis because of their excretory, regulatory, and endocrine functions. The excretory function is initiated by filtration of blood at the glomerulus, and the regulatory function is provided by the tubular segments. Although the prior studies demonstrated that renal cells seeded on biodegradable polymer scaffolds are able to form some renal structures *in vivo*, complete renal function could not be achieved. A subsequent study was designed to create a functional artificial renal unit that could produce urine [71]. Mouse renal cells were harvested, expanded in culture, and seeded onto a tubular device constructed from polycarbonate [72]. The tubular device was connected at one end to a silastic catheter that terminated into a reservoir. The device was implanted subcutaneously in athymic mice. Histological examination of the implanted device demonstrated extensive vascularization as well as formation of glomeruli and highly organized tubule-like structures. Immunocytochemistry using specific antibodies (including osteopontin, alkaline phosphatase, and fibronectin) confirmed the structures. The fluid collected from the reservoir was yellow and contained 66 mg/dL uric acid (as compared with 2 mg/dL in plasma), suggesting that the tubules were capable of unidirectional secretion and concentration of uric acid. The creatinine assay performed on the collected fluid showed an 8.2-fold increase in concentration when compared with serum. These results demonstrated that single cells from multicellular structures can become organized into functional renal units that are able to excrete high levels of solutes in a urine-like fluid [71].

Nuclear transplantation (therapeutic cloning) to generate histocompatible tissues was performed to determine whether renal tissue could be formed using an alternative cell source, and the feasibility of engineering autologous renal tissues *in vivo* using these cloned cells was investigated [38]. Nuclear material from bovine dermal fibroblasts was transferred into unfertilized, enucleated donor bovine oocytes. Renal cells from the cloned embryos were harvested, expanded *in vitro*, and seeded onto 3-D scaffolds. These devices were implanted into the back of the same steer from which the cells were cloned and retrieved

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12 weeks later. This process produced functioning renal units. Urine-like fluid production and viability were demonstrated after transplantation back into the nuclear donor animal. Chemical analysis of the excreted fluid suggested unidirectional secretion and concentration of urea, nitrogen, and creatinine. Microscopic analysis revealed the formation of organized glomeruli and tubular structures. Immunohistochemical and RT-PCR analysis confirmed the expression of renal messenger RNA (mRNA) and proteins, whereas delayed-type hypersensitivity testing and *in vitro* proliferative assays showed that there was no rejection response to the cloned cells. The results of this study indicate that the cloned renal cells were able to organize into functional tissue structures that were genetically identical to the host. Therefore, the generation of immune-compatible cells using therapeutic cloning techniques is technically feasible and could be useful for the engineering of renal tissues for autologous applications.

Although renal cell therapy has been demonstrated in several studies in which implanted, culture-expanded cells show the formation of renal structures, the efficiency of the process of structural reconstitution could not be assessed upon implantation *in vivo*. Reconstitution of renal structure during the culture-expansion stage followed by implantation was proposed to provide a more controlled assessment of renal tissue *in vivo* [10]. A 3-D collagen-based culture system was developed to facilitate the formation of 3-D renal structures *in vitro*. After 1 week of growth, individual renal cells began to form renal structures resembling tubules and glomeruli. Histologically, these structures show phenotypic resemblance to native kidney structures. The reconstituted tubules stained positively for Tamm-Horsfall protein, which is expressed in the thick ascending limb of Henle's Loop and distal convoluted tubules. This study shows that renal structures can be reconstituted in a 3-D culture system that may be used for renal cell therapy applications.

Although many studies have been conducted to achieve improvements in total function, investigations have also been directed toward augmenting selective kidney functions. In one study, cultured renal cells were examined to determine whether EPO-producing cells derived from kidney tissue could be used for the treatment of renal failure-induced anemia [74]. Renal cells from 7-10 day old mice were culture-expanded. The cells were characterized for EPO expression using immunohistochemistry, fluorescence activated cell sorting (FACS), and Western blot analysis at each subculture stage. The levels of EPO expression were analyzed from renal cells incubated under normoxic and hypoxic conditions. The cultured renal cells expressed EPO at each subculture stage tested. Results of this study indicate that EPO-producing cells may be developed as a possible treatment option for anemia that is caused by chronic renal failure.

There has also been considerable progress in the development of stem cell-based therapies for renal failure. For instance, the existence of nontubular cells in the adult mouse kidney that express stem cell antigen-1 (Sca-1) has been reported. This population of small cells includes a CD45-negative fraction that lacks hematopoietic stem cell lineage markers and resides in the renal interstitial space. Moreover, these cells are enriched for ß1-integrin, are cytokeratin negative, and show minimal expression of surface markers that typically are found on bone marrow–derived MSCs. Clonally derived lines can be differentiated into myogenic, osteogenic, adipogenic, and neural lineages. These renal Sca-1 cells were injected directly into the renal parenchyma of C57BL/6 wild-type mice, shortly after ischemic or reperfusion injury. After 1 month, the injected cells had adopted a tubular phenotype and populated the renal tubule after ischemic injury. These adult kidney-derived cells may potentially contribute to kidney repair and may be important in the development of future regenerative medicine strategies [61].

Sagrinati et al [75] demonstrated that parietal epithelial cells (PEC) in the Bowman's capsule exhibit coexpression of the stem cell markers CD24 and CD133 and expression of the stem cell-specific transcription factors Oct-4 and Bml-1. Lineage-specific markers are absent. This population, which was purified from cultured encapsulated glomeruli, revealed self-renewal potential and a high cloning efficiency. Under appropriate culture conditions, individual clones of $CD24^{+}CD133^{+}$ PEC could be induced to generate mature, functional tubular cells with phenotypic features of proximal and distal tubules, osteogenic cells, adipocytes, and neuronal cells. These cells were injected into severe combined immune-deficient (SCID) mice following rhabdomyolysis-induced acute renal failure. This treatment resulted in the regeneration of the tubular structures of different portions of the nephron, and it significantly ameliorated morphologic and functional kidney damage. Results from this study demonstrated the existence of resident multipotent progenitor cells in the adult kidney and suggested a possible therapeutic use for these cells.

SUMMARY

The devastating effects of ESRD, along with the increasing demand for renal transplantation and the critical shortage of donor organs, has accelerated the search for alternative treatment modalities. Recent advances in cell technologies have allowed for development of cell-based approaches for kidney tissue regeneration. Various tissue-engineering and regenerative-medicine approaches that aim to achieve

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functional kidney support have been investigated and are continuously being improved. These approaches seem to hold promise; however, clinical application of these technologies is still distant. Although it has been demonstrated that renal cells are able to reconstitute into functional kidney tissues *in vivo* and that the concept of cell transplantation is possible, numerous challenges must be met in order to translate these techniques into clinical therapies. Some of these challenges include: (1) the generation of a large tissue mass that would augment systemic renal function, (2) the integration of engineered renal tissue that includes adequate vascularization and excretory systems into the host, and (3) the development of reliable renal failure model systems for testing the efficacy of cell-based technologies.

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Conflict of Interest:

S. Agcaoili - no conflict
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REFERENCES

[1] Anderson JK. Surgical Anatomy of the Retroperitoneum, Adrenals, Kidneys, and Ureter. In: Wein AJ, Kavoussi LR, Novick AC, Paritn AW, Peters CA, eds. *Campbell-Walsh Urology*. 9th ed. Philadelphia, PA: Saunders; 2007:3-37.

[2] Levey AS, Eckardt KU, Tsukamoto Y, et al. Definition and classification of chronic kidney disease: a position statement from Kidney Disease: Improving Global Outcomes (KDIGO). *Kidney Int*. 2005;67(6):2089-2100.

[3] Meguid El Nahas A, Bello AK. Chronic kidney disease: the global challenge. *Lancet*. 2005;365(9456):331-340.

[4] Sagrinati C, Ranconi E, Lazzeri E, Lasagni L, Romagnani P. Stem-cell approaches for kidney repair: choosing the right cells. *Trends Mol Med*. 2008;14(7):277-285.

[5] Culleton BF, Larson MG, Wilson PW, Evans JC, Parfrey PS, Levy D. Cardiovascular disease and mortality in a community-based cohort with mild renal insufficiency. *Kidney Int*. 1999;56(6):2214-2219.

[6] Mc Cullough PA. Scope of cardiovascular complications in patients with kidney disease. *Ethn Dis.* 2002;12(4):S3-44-8.

[7] Drey N, Roderick P, Mullee M, Rogerson M. A population-based study of the incidence and outcomes of diagnosed chronic kidney disease. *Am J Kidney Dis.* 2003;42(4):677-684.

[8] Tveit DP, Hypolite IO, Hshieh P, et al. Chronic dialysis patients have high risk for pulmonary embolism. *Am J Kidney Dis*. 2002;39(5):1011-1017.

[9] Eknoyan G, Lameire N, Barsoum R, et al. The burden of kidney disease: improving global outcomes. *Kidney Int*. 2004;66(4):1310-1314.

[10] Joraku A, Stern KA, Atala A, Yoo JJ. In vitro generation of three-dimensional renal sturctures. *Methods*. 2009;47(2):129-133.

[11] Amiel GE, Yoo JJ, Atala A. Renal therapy using tissue-engineered constructs and gene delivery. *World J Urol.* 2000;18(1):71-79.

[12] Chazan JA, Libbey NP, London MR, Pono L, Abuelo JG. The clinical spectrum of renal osteodystrophy in 57 chronic hemodialysis patients: a correlation between biochemical parameters and bone pathology findings. *Clin Nephrol.* 1991;35(2):78-85.

[13] Cohen J, Hopkin J, Kurtz J. Infectious complications after renal transplantation. In: Morris PJ, ed. *Kidney Transplantation: Principles and Practice*. 5th ed. Philadelphia, PA: Saunders; 1994:364-386.

[14] Moe SM, Chertow GM, Coburn JW, et al. Achieving NKF-K/DOQI bone metabolism and disease treatment goals with cinacalcet HCI. *Kidney Int.* 2005;67(2):760-771.

[15] Barreto FC, Barreto DV, Moyses RM, et al. K/DOQI-recommended intact PTH levels do not prevent low-turnover bone disease in hemodialysis patients. *Kidney Int*. 2008;73(6):771-777.

[16] Wolfe RA, Ashby VB, Milford EL, et al. 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(23):1725-1730.

[17] Port FK, Dykstra DM, Merion RM, Wolfe RA. Organ donation and transplantation trends in the USA, 2003. *Am J Transplant*. 2004;4(Suppl 9):7-12.

[18] Ojo AO, Hanson JA, Wolfe RA, Leichtman AB, Agodoa LY, Port FK. Long-term survival in renal transplant recipients with graft function. *Kidney Int*. 2000;57(1):307-313.

[19] Ojo AO, Hanson JA, Meier-Kriesche H, et al. Survival in recipients of marginal cadaveric donor kidneys compared with other recipients and wait-listed transplant candidates. *J Am Soc Nephrol*. 2001;12(3):589-597.

[20] Amiel GE, Yoo JJ, Atala A. Renal tissue engineering using a collagen-based kidney matrix. *Tissue Eng*. 2000;6:685.

[21] Amiel GE, Atala A. Current and future modalities for functional renal replacement. *Urol Clin North Am*. 1999;26(1):235-246.

[22] Perin L, Giuliani S, Jin D, et al. Renal differentiation of amniotic fluid stem cells. *Cell Prolif*. 2007;40(6):936-948.

[23] Kurian MS, Reynolds ER, Humes RA, Klein MD. Cardiac tamponade caused by serous pericardial effusion in patients on extracorporeal membrane oxygenation. *J Pediatr Surg*. 1999;34(9):1311-1314.

[24] Humes HD, Fissell WH, Weitzel WF, et al. Metabolic replacement of kidney function in uremic animals with a bioartificial kidney containing human cells. *Am J Kidney Dis*. 2002;39(5):1078-1087.

[25] Atala A. Advances in tissue and organ replacement. *Curr Stem Cell Res Ther*. 2008;3(1):21-31.

[26] Atala A. Engineering tissues, organs and cells. *J Tissue Eng Regen Med*. 2007;1(2):83-96.

[27] Atala A. Recent applications of regenerative medicine to urologic structures and related tissues. *Curr Opin Urol.* 2006;16(4):305-309.

[28] Atala A, Koh C. Applications of tissue engineering in the genitourinary tract. *Expert Rev Med Devices*. 2005;2(1):119-126.

[29] Atala A. Tissue engineering and regenerative medicine: concepts for application. *Rejuvenation Res*. 2004;7(1):15-31.

[30] Atala A, Koh CJ. Tissue engineering applications of therapeutic cloning. *Annu Rev Biomed Eng*. 2004;6:27-40.

[31] Koh CJ, Atala A. Therapeutic cloning applications for organ transplantation. *Transpl Immunol*. 2004;12(3-4):193-201.

[32] Koh CJ, Atala A. Tissue engineering, stem cells, and cloning: opportunities for regenerative medicine. *J Am Soc Nephrol*. 2004;15(5):1113-1125.

[33] Carley WW, Milici AJ, Madri JA. Extracellular matrix specificity for the differentiation of capillary endothelial cells. *Exp Cell Res*. 1988;178(2):426-434.

[34] Horikoshi S, Koide H, Shirai T. Monoclonal antibodies against laminin A chain and B chain in the human and mouse kidneys. *Lab Invest*. 1988;58(5):532-538.

[35] Humes HD, Cieslinski DA. Interaction between growth factors and retinoic acid in the induction of kidney tubulogenesis in tissue culture. *Exp Cell Res*. 1992;201(1):8-15.

[36] Milici AJ, Furie MB, Carley WW. The formation of fenestrations and channels by capillary endothelium in vitro. *Proc Natl Acad Sci USA.* 1985;82(18):6181-6185.

[37] Schena FP. Role of growth factors in acute renal failure. *Kidney Int Suppl*. 1998;66:S11-S15.

[38] Lanza RP, Chung HY, Yoo JJ, et al. Generation of histocompatible tissues using nuclear transplantation. *Nat Biotechnol.* 2002;20(7):689-696.

[39] Ikarashi K, Li B, Suwa M, et al. Bone marrow cells contribute to regeneration of damaged glomerular endothelial cells. *Kidney Int*. 2005;67(5):1925-1933.

[40] Kale S, Karihaloo A, Clark PR, Kashgarian M, Krause DS, Cantley LG. Bone marrow stem cells contribute to repair of the ischemically injured renal tubule. *J Clin Invest*. 2003;112(1):42-49.

[41] Prockop DJ. Marrow stromal cells as stem cells for nonhematopoietic tissues. *Science*. 1997;276(5309):71-74.

[42] Yokoo T, Ohashi T, Shen JS, et al. Human mesenchymal stem cells in rodent whole-embryo culture are reprogrammed to contribute to kidney tissues. *Proc Natl Acad Sci USA*. 2005;102(9):3296-3300.

[43] Lin F, Moran A, Igarashi P. Intrarenal cells, not bone marrow-derived cells, are the major source for regeneration in postischemic kidney. *J Clin Invest*. 2005;115(7):1756-1764.

[44] Inowa T, Hishikawa K, Takeuchi T, Kitamura T, Fujita T. Isolation and potential existence of side population cells in adult human kidney. *Int J Urol*. 2008;15(3):272-274.

[45] Gupta S, Verfaillie C, Chmielewski D, Kim Y, Rosenberg ME. A role for extrarenal cells in the regeneration following acute renal failure. *Kidney Int*. 2002;62(4):1285-1290.

[46] Ito T, Suzuki A, Imai E, Okabe M, Hori M. Bone marrow is a reservoir of repopulating mesangial cells during glomerular remodeling. *J Am Soc Nephrol*. 2001;12(12):2625-2635.

[47] Iwano M, Plieth D, Danoff TM, Xue C, Okada H, Neilson EG. Evidence that fibroblasts derive from epithelium during tissue fibrosis. *J Clin Invest*. 2002;110(3):341-350.

[48] Lin F, Cordes K, Li L, et al. Hematopoietic stem cells contribute to the regeneration of renal tubules after renal ischemia-reperfusion injury in mice. *J Am Soc Nephrol*. 2003;14(5):1188-1199.

[49] Poulsom R, Forbes SJ, Hodivala-Dilke K, et al. Bone marrow contributes to renal parenchymal turnover and regeneration. *J Pathol.* 2001;195(2):229-235.

[50] Rookmaaker MB, Smits AM, Tolboom H, et al. Bone-marrow-derived cells contribute to glomerular endothelial repair in experimental glomerulonephritis. *Am J Pathol*. 2003;163(2):553-562.

[51] Folkman J, Hochberg M. Self-regulation of growth in three dimensions. *J Exp Med*. 1973;138(4):745-753.

[52] Atala A, Schlussel RN, Retik AB. Renal cell growth in vivo after attachment to biodegradable polymer scaffolds. *J Urol*. 1995;153(Suppl):4.

[53] Atala A, Bauer SB, Soker S, Yoo JJ, Retik AB. Tissue-engineered autologous bladders for patients needing cystoplasty. *Lancet*. 2006;367(9518):1241-1246.

[54] El-Kassaby AW, Retik AB, Yoo JJ, Atala A. Urethral stricture repair with an off-the-shelf collagen matrix. *J Urol.* 2003;169(1):170-173.

[55] Oberpenning F, Meng J, Yoo JJ, Atala A. De novo reconstitution of a functional mammalian urinary bladder by tissue engineering. *Nat Biotechnol*. 1999;17(2):149-155.

[56] Tachibana M, Nagamatsu GR, Addonizio JC. Ureteral replacement using collagen sponge tube grafts. *J Urol.* 1985;133(5):866-869.

[57] Freed LE, Vunjak-Novakovic G, Biron RJ, et al. Biodegradable polymer scaffolds for tissue engineering. *Biotechnology (N Y).* 1994;12(7):689-693.

[58] Hubbell JA, Massia SP, Desai NP, Drumheller PD. Endothelial cell-selective materials for tissue engineering in the vascular graft via a new receptor. *Biotechnology (N Y).* 1991;9(6):568-572.

[59] Mooney DJ, Mazzoni CL, Breuer C, et al. Stabilized polyglycolic acid fibre-based tubes for tissue engineering. *Biomaterials*. 1996;17(2):115-124.

[60] Wald HL, Sarakinos G, Lyman MD, Mikos AG, Vacanti JP, Langer R. Cell seeding in porous transplantation devices. *Biomaterials*. 1993;14(4):270-278.

[61] Dekel B, Burakova T, Arditti FD, et al. Human and porcine early kidney precursors as a new source for transplantation. *Nat Med*. 2003;9(1):53-60.

[62] Steer DL, Bush KT, Meyer TN, Schwesinger C, Nigam SK. A strategy for in vitro propagation of rat nephrons. *Kidney Int.* 2002;62(6):1958-1965.

[63] Rogers SA, Lowell JA, Hammerman NA, Hammerman MR. Transplantation of developing metanephroi into adult rats. *Kidney Int.* 1998;54(1):27-37.

[64] Hammerman MR. Transplantation of embryonic kidneys. *Clin Sci (Lond)*. 2002;103(6):599-612.

[65] Rogers SA, Powell-Braxton L, Hammerman MR. Insulin-like growth factor I regulates renal development in rodents. *Dev Genet*. 1999;24(3-4):293-298.

[66] Rosines E, Sampogna RV, Johkura K, et al. Staged in vitro reconstitution and implantation of engineered rat kidney tissue. *Proc Natl Acad Sci USA*. 2007;104(52):20938-20943.

[67] Rosines E, Schmidt HJ, Nigam SK. The effect of hyaluronic acid size and concentration on branching morphogenesis and tubule differentiation in developing kidney culture systems: potential applications to engineering of renal tissues. *Biomaterials*. 2007;28(32):4806-4817.

[68] Schumacher KM, Phua SC, Schumacher A, Ying JY. Controlled formation of biological tubule systems in extracellular matrix gels in vitro. *Kidney Int.* 2008;73(10):1187-1192.

[69] Hu K, Denk L, de Vries U, Minuth WW. Chemically defined medium environment for the development of renal stem cells into tubules. *Biotechnol J*. 2007;2(8):992-995.

[70] Fung LCT, Elenius K, Freeman M, Donovan MJ, Atala A. Reconstitution of poor EGFr-poor renal epithelial cells into tubular structures on biodegradable polymer scaffold. *Pediatrics.* 1996;98(Suppl):S631.

[71] Yoo J, Ashkar S, Atala A. Creation of functional kidney structures with excretion of kidney-like fluid in vivo. *Pediatrics*. 1996;98(Suppl):S605.

[72] Wald HL, Sarakinos G, Lyman MD, Mikos AG, Vacanti JP, Langer R. Cell seeding in porous transplantation devices. *Biomaterials*. 1993;14(4):270-278.

[73] Yoo JJ, Atala A. A novel gene delivery system using urothelial tissue engineered neo-organs. *J Urol*. 1997;158(3 Pt 2):1066-1070.

[74] Aboushwareb T, Egydio F, Straker L, Gyabaah K, Atala A, Yoo JJ. Erythropoietin producing cells for potential cell therapy. *World J Urol*. 2008;26(4):295-300.

[75] Sagrinati C, Netti GS, Mazzinghi B, et al. Isolation and characterization of multipotent progenitor cells from the Bowman's capsule of adult human kidneys. *J Am Soc Nephrol*. 2006;17(9):2443-2456.