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Cell Approaches in Renal Tissue Engineering

Tamer Aboushwareb
Wake Forest Institute for Regenerative Medicine, Wake Forest University School of Medicine, Winston-Salem, NC, USA
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ABSTRACT

Renal regenerative therapy is an exceedingly complex endeavor due to the multiple functions of the kidney. There is a shortage of donor kidneys and more patients are now progressing to end-stage renal disease, so a significant clinical application from this research is urgently needed. Renal regenerative therapy has great potential for clinical impact in the future through the use of methods described here. Many approaches are currently being researched, but this review focuses on investigations using cellular therapies. The properties of embryonic stem cells, induced pluripotent stem cells (reprogramming), and adult stem cells are compared for their usefulness in regenerative medicine. Methods for identifying the renal progenitor cells that are responsible for generating tubules and glomeruli are discussed, along with the potential contributions of these cells for regeneration and endogenous renal repair. Finally, targeting a single function of the kidney has led to disease-specific cellular therapies for renal dysfunction. Contributions of these therapies to the understanding of stem cells are highlighted. Many methods, techniques, and approaches of regenerative medicine using cells, scaffolds, or a combination of both are being utilized in a variety of projects for kidney regeneration. Therefore, it is not out of the question that we could have a regenerative solution for renal failure in the near future.

INTRODUCTION

Patients with renal disease were the first to benefit from transplantation, because the kidney was the first human organ to be replaced in 1955 [1]. This transplant was performed from one identical twin to another. In the early 1960s, Murray (who later received the Nobel Prize for his work) transplanted a kidney from a nongenetically identical patient. This transplant, which overcame the immunologic barrier, marked a new era in medical therapy and opened the door for the use of transplantation as therapy for different organ systems. Despite this success, the problems that plagued early transplant surgeons still create difficulties today. Immunosuppressive medications are still required to prevent complications, their monitoring and control of graft rejection can be difficult, and there is still a shortage of donor organs. These challenges have led physicians and scientists to seek alternatives in the relatively new fields of tissue engineering and regenerative medicine. Progress in these fields has paved the way for the development of new technologies for renal replacement therapy.

Difficulty in applying regenerative medicine techniques to the kidney is inherent in the complexity of the organ.

KEYWORDS: Kidney; Regenerative medicine; Tissue engineering; Cell therapy; Renal progenitor cells; Metanephros

CORRESPONDENCE: Tamer Aboushwareb, MD, PhD, Wake Forest Institute for Regenerative Medicine and Urology Department, Wake Forest University School of Medicine, Medical Center Boulevard, Winston-Salem, NC 27157 USA (taboushw@wfubmc.edu).

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Abbreviations and Acronyms

AFPS = amniotic-fluid and placental-derived stem
EPO = erythropoietin
ES = embryonic stem
hES = human embryonic stem
iPS = induced pluripotent state
PEC = parietal epithelial cells

Functionally, the kidney is responsible more than waste elimination; it also has endocrine properties. The primary role of the kidney is hemofiltration. The blood is filtered through a complex architectural system. The resulting ultrafiltrate is then concentrated in the renal tubules, which allow for the absorption and secretion of specific ions and water. This process maintains appropriate intravascular concentrations of these components. In addition, the kidney produces erythropoietin, which is responsible for red blood cell production and adequate oxygenation of tissues. Renin is also secreted by the kidney and is a major factor in regulating blood pressure. The kidney activates Vitamin D by converting the circulating 25-hydroxycholecalciferol into 1,25-dihydroxychlecalciferol, and it releases prostaglandins into the circulation. Attempts to completely regenerate renal tissue must provide these important functions in addition to filtration.

Embryologically, the kidney is derived from the integration of several primordial structures. The metanephros is responsible for the development of the proximal section of the nephrons; the ureteric bud forms the collecting ducts and distal structures. The large vessels of the kidney are derived from extrarenal tissues. These divergent embryologic origins converge to produce at least 26 distinct functional cells in the kidney [2]. This heterogeneity creates difficulty with *in vitro* culture and with identifying potential renal stem or progenitor cells.

Despite these hurdles, there are several techniques that are currently being investigated for renal regenerative therapy. Some of these are based on embryology/developmental biology and others depend upon the identification of renal progenitor cells that could eventually be used for clinical applications. Still other techniques for renal regeneration or replacement are based on cell therapies. These include the direct injection of certain cell types as well as the use of biomaterial scaffolds seeded with cells. The present review will focus only on the current status of cell-based therapies in renal regeneration.

DEVELOPMENTAL TECHNIQUES

Integration of New Embryonic Nephrons Into the Kidney

Because it is not practical to regenerate a whole mature kidney in an *in vitro* system, many researchers consider transplantation of a kidney precursor (such as the metanephros) a possible alternative. In an animal study [3], human embryonic metanephros was transplanted to an immune-deficient mouse under the renal capsule. The metanephros that was transplanted into the host kidney developed into a mature kidney. This transplanted metanephros produced urine-like fluid, although the ureter was not developed. From this report, it appears that if we could develop metanephros in an *in vitro* system and transplant this structure, it may be possible to develop small kidneys within the original kidney.

How can metanephros be produced under *in vitro* conditions? Nigam's group [4] reported very interesting results on the propagation of metanephros. They divided the metanephros into mesenchymal tissue and ureteral buds. Then, the mesenchymal tissue and ureteral buds were each cut into thirds and each portion was cultured. After an 8-day culture, each portion of mesenchymal tissue grew to the original size of the mesenchyme. Each portion of mesenchymal tissue was again cut into thirds. After another 8-day culture, each portion had again grown to 3 times its original size. A similar method was applied to the ureteral buds, and the ureteral buds propagated. The investigators then placed the mesenchyme and ureteral bud together in one piece and cultured the tissues *in vitro*; a metanephros-like structure was developed. Based on the results of these reports, it is reasonable to assume that if we can develop mesenchymal cells and ureteric buds from stem cells, we will be able to produce a metanephros in an *in vitro* system.

Growing New Kidneys in Situ

In contrast to the case of rat metanephroi transplanted beneath the renal capsule, metanephroi transplanted into the fold of omentum in a nonimmunosuppressed host rat undergo differentiation and growth in hosts that are unconfined by a tight organ capsule [5]. If transplanted with its ureteric bud attached, the metanephros enlarges and becomes kidney-shaped within 3 weeks. The ureteric bud differentiates into a ureter as it would if left *in situ*. Metanephroi transplanted into the omentum have a normal kidney structure and ultrastructure after development and become vascularized via arteries that originate from the superior mesenteric artery of the hosts [6]. Developed metanephroi have a well-defined cortex and medulla. Mature nephrons and collecting system structures are indistinguishable from those of normal kidneys by light or electron microscopy [6].

Metanephroi transplanted into the omentum survive for as long as 32 weeks after implantation [7]. Ultra-filter inulin infuses into the host's circulation following uretero-ureterostomy between transplant and host, a procedure that can be readily carried out if metanephroi are implanted in close proximity to the host ureter [5,7-10].

STEM CELLS FOR USE IN RENAL TISSUE REGENERATION

Cells used in regenerative medicine techniques can be autologous or heterologous in origin, and they can be either native cells or stem cells. In general, there are 3 broad categories

of stem cells obtained from living tissue that are used for cell therapies. *Embryonic stem (ES) cells* are obtained through the aspiration of the inner cell mass of a blastocyst or, more recently, a single cell from this mass. Fetal and neonatal amniotic fluid and placenta may contain *multipotent cells* that may be useful in cell therapy applications. *Adult stem cells*, on the other hand, are usually isolated from organ or bone marrow biopsies.

Stem cells are defined as having 3 important properties: (1) the ability to self-renew, (2) the ability to differentiate into a number of different cell types, and (3) the ability to easily form clonal populations (populations of cells derived from a single stem cell). Many techniques for generating stem cells have been studied over the past few decades. Some of these techniques have yielded promising results, but others require further research.

Embryonic Stem Cells

Various researchers have shown that ES cells may be useful in renal regenerative medicine [11]. Schulinder et al [12] were able to induce differentiation of human embryonic stem (hES) cells into cells that produce the renal-specific products renin and WT-1 using culture media including Activin-A and hepatocyte growth factor (HGF). Kim and Dressler [13] evaluated whether murine ES cells could be cultured *in vitro* to renal precursor cells and mesoderm. By culturing the ES cells in Activin-A, retinoic acid, and Bmp7, they were able to induce genetic expression of Pax-2 (a marker of intermediate mesoderm from which the renal epithelial cells arise), WT1 (seen in high levels in podocytes), cadherin 6 (an early marker for proximal tubules), and lim1 (seen in intermediate mesoderm). These cells also formed tubule-like structures when introduced *in vivo* to cultured kidney rudiments from 12.5-day embryonic mice.

Vigneau and colleagues [14] showed that ES expressing brachyury (an embryonic nuclear transcription factor that possibly denotes mesoderm) may differentiate into renal progenitor cells in the presence of Activin-A. When these cells were injected into a developing metanephros, they were incorporated into the blastemal cells of the nephrogenic zone. Additionally, the cells were capable of integration into proximal tubules after only a single injection into the kidneys of live newborn mice. At 7 months, there was no evidence of teratoma formation, and the cellular morphology and polarization appeared normal.

In addition to the ethical dilemma surrounding the use of ES cells, their clinical application is limited because they represent an allogenic resource and thus have the potential to evoke an immune response. New stem cell technologies such as somatic cell nuclear transfer and induced pluripotent stem cells promise to overcome this limitation.

Induced Pluripotent Stem Cells (Reprogramming)

Recently, reports of the successful transformation of adult cells into pluripotent stem cells through a type of genetic "reprogramming" have been published. Reprogramming is a technique that involves dedifferentiation of adult somatic cells to produce patient-specific pluripotent stem cells. The advantage of this technique is that it obviates the need for creation of embryos. Cells generated by reprogramming would be genetically identical to the somatic cells (and thus, the patient who donated these cells) and would not be rejected. Takahashi and Yamanaka [15] initially discovered that mouse embryonic fibroblasts (MEFs) and adult mouse fibroblasts could be reprogrammed into an "induced pluripotent state (iPS)". These iPS cells were capable of immortal growth that was similar to the self-renewing characteristics of ES cells, expressed genes specific for ES cells, and generated embryoid bodies *in vitro* and teratomas *in vivo*. When iPS cells were injected into mouse blastocysts, they differentiated into several cell types. However, although iPS cells selected in this way were pluripotent, they were not identical to ES cells. Unlike ES cells, chimeras made from iPS cells did not result in full-term pregnancies. Gene expression profiles of the iPS cells showed that they possessed a distinct gene expression signature that was different from that of ES cells. In addition, the epigenetic state of the iPS cells was somewhere between that found in somatic cells and that found in ES cells, suggesting that the reprogramming was incomplete.

Wernig et al [16] significantly improved these results in July 2007. In this study, DNA methylation, gene expression profiles, and the chromatin state of the reprogrammed cells were similar to those of ES cells. Teratomas induced by these cells contained differentiated cell types representing all mesoderm, ectoderm, and endoderm. Most importantly, the reprogrammed cells from this experiment were able to form viable chimeras and contribute to the germ line like ES cells, suggesting that these iPS cells were completely reprogrammed.

It has recently been shown that reprogramming of human cells is possible. Takahashi et al [17] generated human iPS cells that are similar to hES cells in terms of morphology, proliferation, gene expression, surface markers, and teratoma formation. Thompson's group [18] showed that retroviral transduction of the stem cell markers *OCT4, SOX2, NANOG*, and *LIN28* could generate pluripotent stem cells. However, in both studies, the human iPS cells were similar but not identical to hES cells. Recent efforts by several researchers have demonstrated successful creation of iPS in more than one animal model [19,20]. The porcine model was most important of those developed, due

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to its similarity to the human kidney. The investigators each found retroviral transfection of cells with expression of stem cell markers. These are exciting potential preclinical models for future therapeutic direction [21]. Our limited understanding of the mechanism underlying reprogramming currently limits the clinical applicability of the technique, but the future potential is quite exciting.

Amniotic-Fluid and Placenta-Derived Stem Cells

The amniotic fluid and placental membrane contain a heterogeneous population of cell types derived from the developing fetus [22,23]. Cells found in this heterogeneous population include mesenchymal stem cells [24,25]. In addition, the isolation of multipotent human and mouse amniotic-fluid and placental-derived stem (AFPS) cells that are capable of extensive self-renewal and give rise to cells from all 3 germ layers was reported in 2007 [26]. AFPS cells represent approximately 1% of the cells found in the amniotic fluid and placenta. The undifferentiated stem cells expand extensively without a feeder-cell layer and double every 36 hours. Unlike human embryonic stem cells, the AFPS cells do not form tumors *in vivo*. Lines maintained for over 250 population doublings retained long telomeres and a normal complement of chromosomes. AFPS cell lines can be induced to differentiate into cells representing each embryonic germ layer, including cells of adipogenic, osteogenic, myogenic, endothelial, neural-like, and hepatic lineages. In addition to the differentiated AFPS cells expressing lineage-specific markers, such cells can have specialized functions. Cells of the hepatic lineage secreted urea and a-fetoprotein, while osteogenic cells produced mineralized calcium. In this respect, they meet a commonly accepted criterion for multipotent stem cells without implying that they can generate every adult tissue.

AFPS cells represent a new class of stem cells with properties somewhere between those of embryonic and adult stem cell types. They are probably more agile than adult stem cells, but less agile than ES cells. Unlike embryonic and induced pluripotent stem cells, however, AFPS cells do not form teratomas; if preserved for self-use, they avoid the problems of rejection. The cells could be obtained either from amniocentesis or chorionic villous sampling in the developing fetus, or from the placenta at the time of birth. They could be preserved for self-use and used without rejection, or they could be banked. A bank of 100,000 specimens could potentially supply 99 percent of the population in the United States with a perfect genetic match for transplantation. Such a bank may be easier to create than a bank from other cell sources, because there are approximately 4.5 million births per year in the USA.

Since the discovery of the AFPS cells, other research groups have published information about the potential of the cells to differentiate to other lineages such as cartilage [27] and lung [28]. Perin and colleagues [29] investigated whether or not AFPS could differentiate into renal structures by labeling human cells and injecting them into murine embryologic renal tissues. The anlagen were cultured *in vitro* using a novel method to allow embryologic survival through 10 days in culture. The AFPS integrated in renal structures including C and S bodies. Reverse transcriptase polymerase chain reactions (rtPCR) demonstrated zona-occludens 1, claudin, and glial-derived neurotrophic factor, all of which are early markers for renal differentiation. They were not seen in AFPS that was not injected into the anlagen. This study demonstrates that AFPS can differentiate into renal lineage when cultured *in vitro* with renal precursors. Although these results are promising, further *in vivo* studies and functional assays are required prior to clinical applications.

Adult Stem Cells

Adult stem cells, especially hematopoietic stem cells, are the best understood cell type in stem cell biology [30]. However, adult stem cell research remains an area of intense study, because their potential for therapy may be applicable to a myriad of degenerative disorders. Within the past decade, adult stem cell populations have been found in many adult tissues other than the bone marrow and the gastrointestinal tract, including the brain [31,32], skin [33], and muscle [34]. Many other types of adult stem cells have been identified in organs all over the body and are thought to serve as the primary repair entities for their corresponding organs [35]. The discovery of such tissue-specific progenitors has opened up new avenues for research.

A notable exception to the tissue-specificity of adult stem cells is the mesenchymal stem cell, also known as the multipotent adult progenitor cell. This cell type is derived from bone marrow stroma [36,37]. Such cells can differentiate *in vitro* into numerous tissue types [38,39] and can also differentiate developmentally if injected into a blastocyst. Multipotent adult progenitor cells can develop into a variety of tissues including neuronal [40], adipose [34], muscle [34,41], liver [42,43], lungs [44], spleen [45], and gut tissue [37], but notably not bone marrow or gonads. There has been considerable research into the identification, characterization, and expansion of renal multipotent progenitor cells.

RENAL PROGENITOR CELLS

Identification of a single progenitor cell responsible for generating all of the cell types in the kidney is unlikely due to the diverse nature of the kidney. However, because the majority of renal dysfunction results from disorders of either

the tubules or the glomeruli, identifying the progenitor cells responsible for generating these structures could produce the most significant clinical impact. One method for identifying progenitor cells includes the recognition of specific cell surface markers. Gupta et al [46] and Bussolati and colleagues [47] chose to determine whether or not CD133+ cells reside in the adult human renal parenchyma. CD133 is expressed in hematopoetic and proginator cells and in the embryonic kidney. These researchers observed that a CD133+ population was present and comprised approximately 0.8% of the total cell population within the renal cortex. The cells expressed Pax-2 but not CD-45 (a marker of the hematopoetic lineage), cKit, and CD90 (stem cell markers) [47]. *In vivo*, these cells formed tubular-like structures following implantation into immunocompromised mice. Following enzymatic digestion of rat kidneys, Plotkin and associates [48] identified a single clone that expressed Sca-1, CD44, CD34, the transcription factors Pod-1, BF-2, and receptors for sonic hedgehog, BMP, and retinoic acid. When these cells were injected into the subcapsule following an ischemia/reperfusion injury, the isolated cells integrated into the peritubular capillaries and periphery of the papillae. Gupta et al [46] enzymatically digested 4-week-old rat kidneys and cultured the resulting cellular suspension using culture methods that are similar to those used for bone marrow-derived cells. They identified a population of cells that were capable of more than 200 population doublings without senescence. These cells expressed vimentin, Oct-4, and Pax-2, but did not express markers of either MHC class or markers of differentiated cells. When labeled cells from this study were reintroduced *in vivo*, they demonstrated integration into the renal architecture but did not improve renal function following ischemia/reperfusion injury. The identification of renal cells, which can integrate into the renal parenchyma, has implications for future therapies based on the replacement of nephrons. Integration of nephrons is especially promising for patients with progressive renal dysfunction to prevent end-stage renal disease.

Podocytes also present a novel model for understanding and identifying potential renal progenitor cells. Podocytes are postmitotic cells that lack the ability to reproduce but are routinely found in the urine, even in healthy individuals [49]. The number of cells lost in the urine exceeds the number that would be expected for renal survival of 80 years. Therefore, it has been hypothesized that an inherent population of progenitor cells resides in the kidney and is capable of regenerating podocytes. Several groups have investigated the role of progenitor cells that may be involved in podocyte regeneration. Sagrinati and colleagues [50] found a population of CD133+CD24+ cells that were localized opposite the vascular pole of Bowman's capsule. These parietal epithelial cells (PEC) were between 0.5% and 4% of the total cellular population. They were maintained in up to 90 population doublings and expressed Oct-4 and BmI-1. When introduced *in vitro* following glycerol-induced rhabdomyolysis acute renal injury, these cells attenuated renal injury as measured by lower BUN levels 7-10 days following injection.

Further investigation of PECs by Ronconi et al [51] identified 3 subpopulations of these cells. A subset of undifferentiated cells expressing only CD133/24+ was located at the urinary pole. A second transitional population of cells expressing CD133/24 and nestin, complement receptor-1 (CR1), and podocalyxin (PDX) (all podocyte markers) was located between the urinary and vascular poles. Continuous with the podocytes was a subset of cells that no longer expressed CD133/24, but were positive for nestin, CR1, and PDX. *In vitro*, the only cells capable of differentiating into tubular cells were those that were CD133/24+ but not PDX+. To evaluate *in vivo* properties of these cells, a focal segmental glomerulosclerosis (FSGS) model of renal dysfunction was created with adriamycin infusion. The CD133/24+PDX- cells were able to reduce albuminuria and decrease glomerular and tubular quantitative injury in this model [51]. In another series of experiments, Appel et al [52] evaluated the CD133+ PEC and concluded that the PECs were capable of migration along the glomerular tuft to replace podocyte populations. They examined the cells at the base of the vascular pole adjacent to the podocytes and noted that only these cells could be stained with both PEC markers (claudin-1) and podocyte-specific markers of nestin, Dipeptidyl peptidase IV (DPP IV), or aminopeptidase A. A PEC-specific promotor (podocalyxin) was identified that could be used to trace the migration of cells. This experiment pointed to recruitment of PEC by podocytes.

Identification of renal progenitor cells is a substantial step forward in the understanding of the inherent regenerative capacity of the kidney. The ability to identify the subset of cells responsible for endogenous renal repair and expand these cells *in vitro* could have future therapeutic implications. However, caution must be taken in referring to these identified cells as renal stem cells. Currently, no group has demonstrated a population of cells that satisfy the strict definition of a *stem cell*, which includes the ability to form clonal populations *in vitro*. In addition, this technology still needs refinement prior to clinical use.

CELLULAR-BASED THERAPIES

The functional heterogeneity and complex cellular architecture within the kidney present many challenges to the development of cellular therapies. These include the need to ensure precise

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delivery of the cells (which allows homing to the necessary location) and the need to define exactly what type of cell should be delivered. Some groups have evaluated the possibility of using stem cells from various lineages; others have focused on the use of differentiated renal cells to improve function.

Isolation of a single cell type for reintroduction to the kidney may be an initial step in providing cellular therapies for renal dysfunction. One example of this type of therapy is the treatment of anemia associated with chronic renal dysfunction. Currently, patients with end-stage renal disease often require recombinant erythropoietin (EPO) injection in order to increase red blood cell counts. Recent research into alternatives to these injections has presented a novel platform for future clinical applications for cellular therapy. Isolation and culture of erythropoietin-producing renal cells has been described by Aboushwareb et al [53]. Over the past 2 years, characterization of these cells has been done in rodents (Figure 1), pigs, and humans. Isolation and expansion of these cells may provide an ideal substitute for the current therapies using repeated recombinant EPO injections. These cells have also demonstrate increased EPO expression when exposed to hypoxic environments (Aboushwareb et al, unpublished data). The data in Figure 2 show that these cells have the ability to regulate their EPO expression *in vitro* according to the surrounding environment. These cells represent a potential lineage for future treatments of renal dysfunction-associated anemia. In another study on this topic, Bathlolomew and colleagues [54] transfected baboon mesenchymal stem cells (MSC) with human EPO vectors and evaluated whether or not these cells were capable of *in vivo* EPO production. These cells could successfully produce EPO for up to 28 days in mice with severe combined immune deficiency (SCID) and up to 137 days in baboons. Targeting of anemia associated with renal dysfunction or other singular functions of the kidney may provide pathways to introduce cellular therapies for renal dysfunction.

Targeting specific cells in the nephron could provide disease-specific cellular therapy. Patients may have either tubular dysfunction or podocyte dysfunction. Thus, each patient requires specific therapy based on pathology. Han and colleagues [55] found that primary rabbit proximal tubule cells are able to produce functional tubular units. Following enzymatic digestion of the cortex and removal of glomeruli,

Figure 1. Characterization of Erythropoietin-Expressing Cells in Primary Renal Culture.
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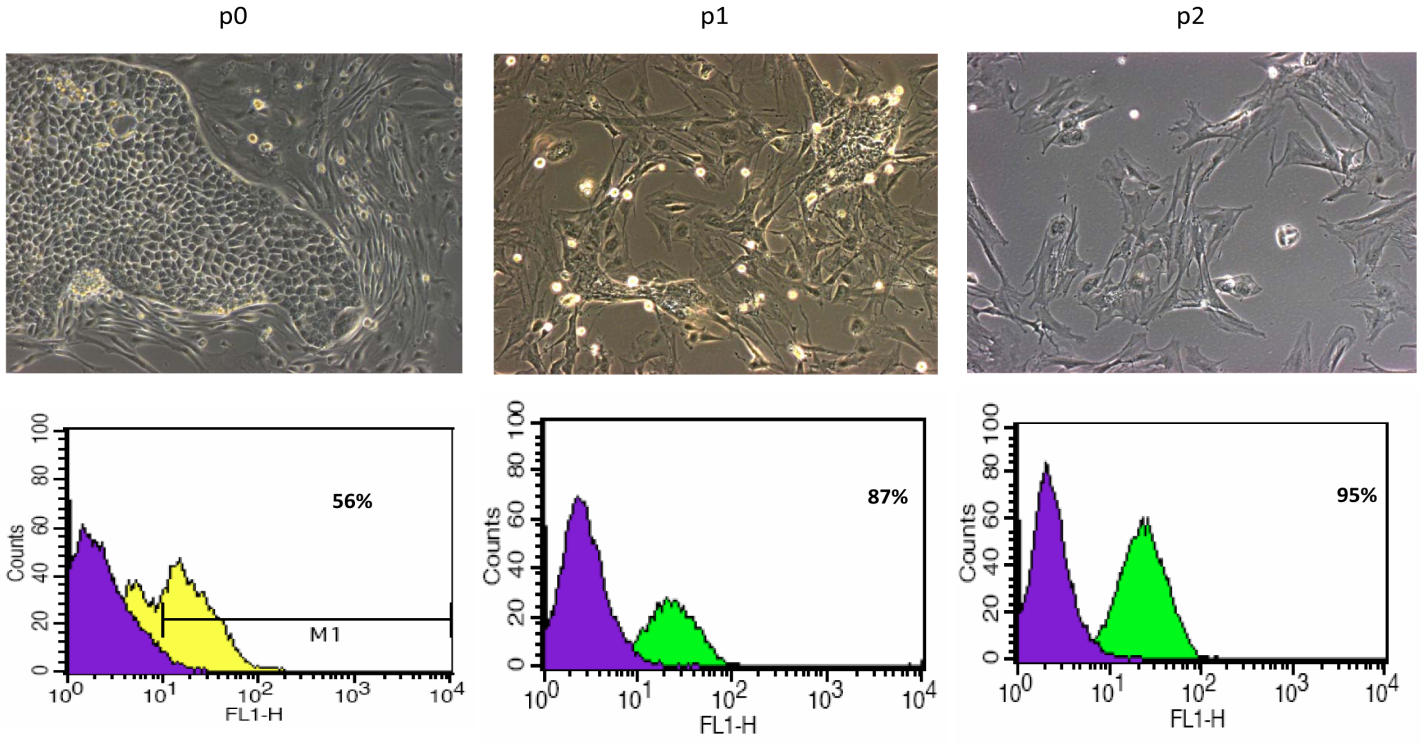

Figure 2. Effect of Hypoxia and Normal Conditions on Erythropoietin Expression.
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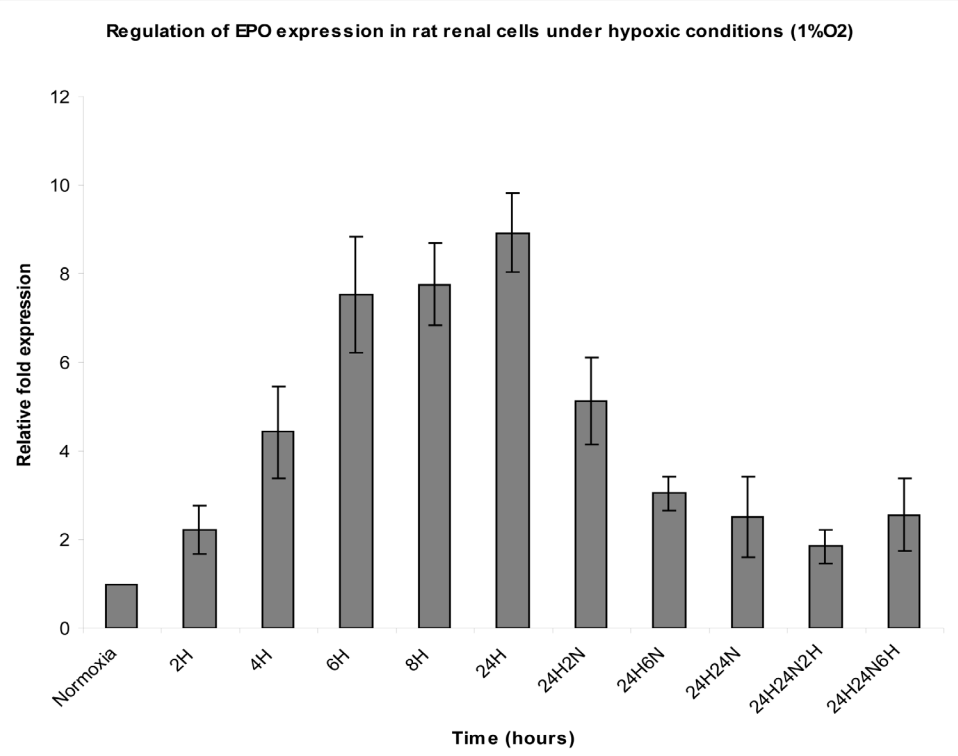

Rat renal cells (p0) were placed in a 1% O_2 hypoxic chamber (H) for up to 24 hours (24H). Cells were returned to normal conditions (N) for 2, 6, and 24 hours and then placed back in the hypoxic chamber for 2 to 6 hours. All samples were processed for qPCR analysis of total RNA for EPO expression. Data are expressed as mean ± SEM (N=5).

tubules were formed *in vitro* with luminal formation. Function was demonstrated with Lucifer Yellow, a fluorescent substrate of the p-aminohippurate (PAH) transport system transport. In patients with tubular dysfunction, introduction of cultured tubules could direct therapy at the specific pathology of the disease.

Development of heterogenous populations of renal cells could also represent a potentially viable option for cellular therapy of renal disease. Joraku et al [56] developed an *in vitro* method for cultivation of mixed renal cells that allowed the development of tubular structures. This technique involves digestion of the entire murine kidney, followed by cultivation of the resulting cells on rat-tail type-1 collagen. Upon histologic examination, cells from the thick ascending loop of Henle stained positive for Tamm-Horsfall protein. This work has been continued by Souza et al (unpublished data). They demonstrated the same phenomenon of self-assembly with isolated human kidney cells. These results highlight the potential of isolated expanded kidney cells to reassemble functional renal units within diseased kidneys. However, the published reports of reintroduced adult renal cells have not yet demonstrated improvements in renal function from this treatment, nor has the barrier of immune recognition been overcome [57].

CONCLUSIONS

End-stage renal disease represents an enormous burden on health care systems around the globe. Potential cell therapy approaches currently being investigated in the area of renal regeneration are reviewed in this article. Advances in stem cell technologies and proper control of their differentiation potential are greatly needed for the advancement of the entire field. Recently, most investigators have turned to induced pluripotent stem cells as the most attractive approach for stem cell therapies. Advancement in this field will surely be linked to advancement in many medical therapies, whether using cell therapies or total organ reconstruction. Tissue engineering of the kidney is one of the primary topics currently being

investigated in the field of regenerative medicine. Although the complexity of the kidney and its structure create a unique challenge for researchers, there is marked optimism for success in kidney function improvement in the entire field.

Conflict of Interest: none declared.

GLOSSARY

Acellular matrix: collagen-rich scaffolds prepared by removing all cellular components from a donor tissue.

Adult stem cell: an undifferentiated cell found in postnatal tissues that can self-renew and produce progeny that can differentiate into the various cell types that comprise the tissue.

Amniotic fluid stem (AFS) cell: a multipotent cell type found in the amniotic fluid surrounding an embryo; AFS cells may be differentiated to hepatic, pancreatic, renal, cardiac, osteogenic, and chondrogenic lineages, among others.

Distal tubule: the final segment of the nephron in the kidney, located between the loop of Henle and the collecting duct; it regulates serum concentrations of potassium, sodium, and calcium as well as pH.

Embryonic stem (ES) cell: a broadly multipotent cell type found in the inner cell mass of a blastocyst; ES cells can differentiate into nearly every cell type in the body.

Erythropoietin: a glycoprotein cytokine hormone produced by the peritubular capillary endothelial cells in the kidney; it controls erythropoiesis (red blood cell production).

Glomerulus: the basic filtration unit of the kidney; it is composed of a capillary tuft surrounded by Bowman's capsule and performs the first step in filtering blood to form urine. The glomerular filtration rate (GFR) is the rate at which blood is filtered through all of the glomeruli and thus the measure of the overall renal function.

Loop of Henle: the portion of the nephron that leads from the proximal straight tubule to the distal convoluted tubule; it functions to create concentrated urine for excretion by forming an area of high sodium concentration deep in the medulla of the kidney, so that water present in the filtrate flows through aquaporin channels out of the collecting duct and is reabsorbed.

Metanephros: an embryonic structure that becomes the permanent kidney in reptiles, birds, and mammals; it develops by the 10th week of gestation in human embryos.

Nephron: the basic structural and functional unit of the kidney; it regulates the concentration of water and soluble substances like sodium salts by filtering the blood, reabsorbing what is needed, and excreting the rest as urine. It also regulates blood volume and blood pressure, controls levels of electrolytes and metabolites, and regulates blood pH. In humans, a normal kidney contains 800,000 to 1,000,000 nephrons.

Podocyte: a cell with branching tentacle-shaped extensions that constitutes the barrier through which blood is filtered in the glomerulus of the kidney.

Proximal tubule: the portion of the nephron of the kidney that leads from Bowman's capsule to the loop of Henle; it regulates the pH of renal filtrate by exchanging hydrogen ions in the interstitium for bicarbonate ions in the filtrate. It is also responsible for secreting organic acids such as creatinine and other bases into the filtrate.

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