Concise Review: Stem/Progenitor Cells for Renal Tissue Repair: Current Knowledge and Perspectives

SHIKHAR AGGARWAL,* ALDO MOGGIO,* BENEDETTA BUSSOLATI

Key Words. Kidney • Marrow stromal stem cells • Renal • Progenitor cells

ABSTRACT

The kidney is a specialized low-regenerative organ with several different types of cellular lineages; however, the identity of renal stem/progenitor cells with nephrogenic potential and their preferred niche(s) are largely unknown and debated. Most of the therapeutic approaches to kidney regeneration are based on administration of cells proven to enhance intrinsic reparative capabilities of the kidney. Endogenous or exogenous cells of different sources were tested in rodent models of ischemia-reperfusion, acute kidney injury, or chronic disease. The translation to clinics is at the moment focused on the role of mesenchymal stem cells. In addition, bioproducts from stem/progenitor cells, such as extracellular vesicles, are likely a new promising approach for reprogramming resident cells. This concise review reports the current knowledge about resident or exogenous stem/progenitor populations and their derived bioproducts demonstrating therapeutic effects in kidney regeneration upon injury. In addition, possible approaches to nephrogenesis and organ generation using organoids, decellularized kidneys, and blastocyst complementation are surveyed.

INTRODUCTION

The intrinsic capacity for tissue repair and regeneration necessary to reacquire functionality after ischemic, toxic, or inflammatory insults is limited in the mammalian kidney. Acute kidney injury remains a major cause of in-hospital morbidity and mortality, despite the supply of supportive care. In addition, partial/ altered remodeling of the damaged tissue, as a consequence of repeated acute injuries, may lead to tissue fibrosis and possibly to organ failure. Regeneration of injured portions of the tubules may occur through mechanisms that rely on the intrinsic ability of resident progenitors or dedifferentiated resident cells to proliferate and substitute the damaged tissue. Approaches based on stem/progenitor cell administration or pharmacological modulation can be viewed as a promising option able to foster this intrinsic renal regeneration (Fig. 1). At variance with this, in chronic renal failure, in which most of the functioning tissue is lost, a stem cell-based therapy would need to regenerate whole new nephrons. Although in lower species, such as in fish and amphibians, nephrogenesis may occur, this mechanism is lost in mammals. Attempts to generate new nephrons are currently under investigation using organ culture and decellularized renal tissue (Fig. 1).

In this review, we present data on the role of progenitor/stem cells in renal regeneration and on the perspectives of their clinical application in short to medium term, discussing the advantages, limitations, and future challenges related to this rapidly evolving field. Three areas of interest are covered: (a) the possible use of mesenchymal stem cells (MSCs) of different origins and their possible substitution with MSC-derived bioproducts, such as microvesicles and exosomes; (b) the involvement of resident progenitors cells in renal regeneration; and (c) the recent advances in nephrogenesis and kidney organ reconstruction using organoids, decellularized kidneys, and blastocyst complementation. These different approaches are shown in Table 1.

CELL THERAPY WITH MSCS

Experimental Models, Sources, and Administration

MSCs are the most established type of stem cells to support renal repair and the most advanced in clinical development. They can typically be isolated from bone marrow (BM), but other sources of cells sharing similar phenotype and properties have been reported, such as adipose tissue and neonatal birth-associated tissues (umbilical cord, placenta, and amniotic fluid) [1]. Although MSCs are able to differentiate in multiple cell lines, including the renal one, it is now accepted that their behavior in vivo after therapeutic administration is mainly restricted to mesodermal tissues [2]. Despite the lack of direct nephrogenic...
Regarding chronic renal failure, some conflicting results were reported on the effect of MSCs in models of renal fibrosis. MSCs were shown to exert a beneficial effect in rat models of fibrosis induced by 5/6 nephrectomy or by unilateral ureteral obstruction [11, 12]. Furthermore, MSCs reduced fibrosis in a porcine model of renal artery stenosis [13]. The effect of MSCs in preventing the renal fibrotic processes may be mainly related to protection from injury development, rather than to regeneration of the damaged tissue, as shown in a rat model of chronic allograft nephropathy [14]. In contrast, no clinical response was observed when cryopreserved, allogeneic adipose MSCs were tested in cats with spontaneous kidney failure [15].

The possible use of BM-MSCs from patients with end-stage kidney disease may also present some limitation. Uremia was reported to affect the cell functional properties, thus possibly preventing the possible use of autologous BM-MSCs [16, 17]. At variance with this, Reinders et al. [18] showed that BM-MSCs from patients with end-stage renal disease were suitable for autologous therapy. This is of particular importance as these cells are the only ones, at present, used in clinical trials. Interestingly, it was recently shown by the same group that MSCs derived from adipose tissue were not affected by uremia [19] and provided renal protection in a model of chronic renal failure and fibrosis [20].

MSCs isolated from adipose tissue also offer advantages compared with BM-MSCs in terms of collection, tissue processing, and clinical complications. In fact, the collection of bone marrow aspirate is a painful and invasive procedure, can be accompanied by the risk of infection, and sometimes yields low numbers of MSCs after processing [1].

The method of administration of MSCs may influence their localization into damaged tissues, as MSCs are prone to being trapped in the tissue capillaries of lung, spleen, and liver [21]. The administration route through the distal thoracic aorta (from the left carotid or femoral artery) may provide benefits in comparison with intravenous administration, as it avoids the pulmonary circulation obstruction. However, the intravascular administration of MSCs may lead to prothrombotic events [22]. For these reasons, different routes of MSC administration have been verified in animal models of kidney diseases. Direct MSC implantation into the renal parenchyma [23] and injection into the renal subcapsular region [24] have been proven to be efficient. In a recent work, Zhuo et al. [25] compared the biodistribution of MSCs by bioluminescence imaging tracing after their injection in the tail vein or in the renal artery in a rat model of kidney injury. In this study, even though MSCs were entrapped in lung, liver, and spleen, both administrations showed improvement of the damage, suggesting that the paracrine effect exerted by MSCs localized in distant sites could be responsible for their beneficial effects [25]. Interestingly, a meta-analysis of experimental models of renal failure in mice and rats (21 studies analyzed) showed that arterial delivery of MSCs caused greater reduction in elevated creatinine compared with intrarenal delivery and intravenous injection [26].

MSCs: Clinical Trials

Based on their anti-inflammatory and tissue regenerating properties [27, 28], BM-MSCs are currently being used in clinical trials for the regeneration and repair of different tissues (kidney, liver, lung, and heart) (see ClinicalTrials.gov, http://www.clinicaltrials.gov). In nephrology, the first phase I clinical trial evaluated the effect of allogeneic MSC administration to open-heart surgery patients at high risk of acute renal failure [29]. The results demonstrated the safety and efficacy of MSC therapy, as no treated patient required hemodialysis. Two subsequent trials, performed to evaluate the effect of MSCs in renal transplant patients, have recently been published [30, 31]. Although the protolerogenic effects of MSCs in organ transplants are not discussed here, as they are beyond the scope of this review,

Figure 1. Schematic illustration depicting the different approaches to renal repair and regeneration. Therapeutic approaches using stem/progenitor cells, bioproducts, or drugs can be viewed as a promising option able to foster an intrinsic renal regeneration. The possibility of regenerating whole new nephrons (i.e., nephrogenesis) is required when the functioning renal tissue is lost and renal progenitors are exhausted. Renal organoids or bioengineered kidneys could generate a critical mass of functioning renal tissue, possibly exploiting artificial devices and decellularized kidneys. Abbreviations: EV, extracellular vesicle; iPS, induced pluripotent stem; MSC, mesenchymal stem cell.
these clinical trials have shown that MSCs were safe and provided an early recovery of graft function. However, early post-transplant MSC infusion was found to be possibly associated with acute graft dysfunction (one patient out of two in the trial) because of MSC localization into the graft, called engraftment syndrome [30]. This negative effect, accompanied by neutrophil recruitment and complement C3 deposition, was possibly attributable to the inflammatory milieu occurring after kidney transplantation, leading to altered MSC functions [32].

Recently, a phase I study of autologous BM-MSCs was set up for the treatment of allograft rejection after renal transplantation from living donors [33]. In this work, MSCs were given intravenously, at intervals of 7 days, to patients when a protocol renal biopsy at 1 or 6 months showed signs of rejection and/or an increase in interstitial fibrosis or tubular atrophy. Despite the low number of patients treated (n = 6), two renal biopsies after MSC treatment showed resolution of interstitial fibrosis and tubular atrophy and reduction of cell infiltrates, suggesting a regenerative and anti-inflammatory effect of the cell therapy on the renal tissue [33]. The negative short-term effect of MSC administration referred to the engraftment syndrome was not observed in this trial. This could have been due to a late timing of MSC administration, beyond 6 months, after transplantation [33].

**Therapeutic Effect of MSC-Derived Growth Factors and Extracellular Vesicles**

It has been established that MSCs interact with resident cells in endocrine and paracrine ways, releasing growth factors, cytokines, prostaglandins, enzymes, or extracellular vesicles [27, 34, 35] (Fig. 2). Exploiting the mechanisms of the paracrine effect of MSCs, MSC-derived products themselves can be explored as a therapeutic tool. MSC-derived conditioned medium containing growth and anti-inflammatory factors, such as hepatocyte growth factor, vascular endothelial growth factor, and insulin-like growth factor (IGF), was shown to allow kidney recovery after injury, in analogy with cell injection [34, 37].

The cell activity of MSCs was also compared with that of MSC-derived extracellular vesicles (EVs). These membrane-covered particles released by cells can be subdivided on the basis of size, origin, and protein markers [38]. Exosomes have a mean

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**Table 1. Different approaches for renal tissue repair using stem/progenitor cells and organ generation**

| Therapeutic approach                   | Experimental model                                      | Remarks                                               | References |
|---------------------------------------|--------------------------------------------------------|-------------------------------------------------------|------------|
| Stem/progenitor cells                 |                                                        |                                                       |            |
| Rodent cells                          |                                                        |                                                       |            |
| MSCs                                  | Glycerol/cisplatin/I-R injury/5/6 nephrectomy          | Improved survival and renal function/prevented chronic damage | [3, 4, 6, 11, 14] |
| Nontubular sca1 <sub>lin</sub> cells   | I-R injury                                             | Tubular integration                                   | [50]       |
| Renal MSCs (pericytes)                | Isolated from adult murine kidney                      | Differentiation into rennin-producing cells            | [68]       |
| NFATc1 <sub>1</sub> cells             | Mercuric chloride induced injury                        | Proximal tubule repair                                | [52]       |
| Human cells                           | Glycerol/cisplatin injury in SCID mice                  | Improved survival and renal function                  | [7, 9, 36] |
| MSCs                                  | Clinical trials                                        | Phase I trials, some inflammatory concerns (engraftment syndrome) | [30–33] |
| Cortical CD133 <sub>+</sub> cells      | Glycerol induced injury in SCID mice                    | Tubular integration, epithelial and endothelial differentiation | [56]       |
| CD133"CD24-" cells                   | Glycerol and adriamycin-induced injury in SCID mice     | Tubular and glomerular integration and regeneration    | [57, 64]   |
| Renal MSCs (pericytes)                | CD133<sub>CD146</sub> cells in human glomerulus        | Self renewal, clonogenicity, multipotency              | [66]       |
| Stem cell bioproducts                 |                                                        |                                                       |            |
| MSC-derived EVs                       | Glycerol/cisplatin/I-R induced injury                   | Improved renal function/reduced lethality/prevented chronic damage | [39, 40] |
| MSC-derived cytokines/growth factors  | Acute tubular injury/cisplatin injury                   | Improved renal function/reduced lethality             | [34, 37]   |
| Organ generation                      |                                                        |                                                       |            |
| Metanepheric mesenchyme/Wolffian duct coculture | In vitro system                                       | Formation of nascent nephrons                         | [71, 72]   |
| Artificial 3D scaffolds               | Cells coated on supporting matrix                      | Formation of tubules and glomerulus-like structures    | [73]       |
| Dissociation/reassociation without scaffolds | Organoids under renal capsule of athymic mice          | Functional engineered nephron                         | [74–76]    |
| Decellularization/acellularization    | Rat, rhesus monkey, porcine kidneys                     | Cell engraftment and differentiation/generation of functional kidneys | [77–80]    |
| ES cells                              | Rat/mouse                                              | Organoid generation/differentiation into epithelial and tubular cells | [83, 85, 86] |
| iPScells                              | Murine/human renal cells to generate iPScells          | iPScell differentiation into podocytes                 | [90–94]    |
| De novo blastocyst complementation    | Injection into the blastocysts of an anephric mouse    | Repopulate the empty niche of the kidney with donor iPScells | [97]       |

Abbreviations: 3D, three-dimensional; ESC, embryonic stem cell; EV, extracellular vesicle; iPSc, induced pluripotent stem; I-R, ischemia-reperfusion; MSC, mesenchymal stem cell; SCID, severe combined immunodeficient.
size of 100 nm and are released from intracytoplasmatic multivesicular bodies. Microvesicles are instead released by membrane budding and have a larger size, ranging from 100 to 1,000 nm. Regardless of the differences in origin and size, EVs are characterized by their content in proteins, receptors, mRNAs, and microRNAs, which interact with the target cells, causing a biological effect [38].

MSC-derived EVs injected in veins have been shown to display a therapeutic role, improving the renal function in a glycerol-induced acute injury model and preventing lethality in a cisplatin-induced acute renal damage, as reported for MSCs themselves [39–41]. In particular, the effect of EVs collected from an overnight MSC culture was comparable to that obtained by injecting the same number of cells [39]. The effect of EVs is considered the result of EV uptake by damaged human tubular epithelial cells, as shown in vivo a few hours after administration using labeled EVs [39], and was shown to be related to the transfer of mRNA and microRNAs [39–41]. In fact, when conditioned medium or EVs from MSCs were treated with RNase to degrade RNA, the therapeutic effects were blunted. Indeed, human proteins were shown in the renal tissue of mice injected with human MSC-derived vesicles [39]. Moreover, a study by Tomasoni et al. [42] proved that MSC-derived EVs induced proliferation of cisplatin-damaged tubular cells by transferring the mRNA for IGF-1 receptor. In addition, repeated injections of MSC-derived EVs prevented renal fibrosis from occurring after ischemia-reperfusion injury [43].

All these preclinical data open new perspectives for the possible use of EVs or conditioned medium in clinical trials as in the case of MSCs. Although further studies regarding the safety, effectiveness, and biodistribution of MSC-derived EVs are required, they appear to be an alternative therapeutic approach to cells with several advantages. The potential risks associated with a stem cell therapy, such as maldifferentiation or tumor development, could be avoided. Cryopreserved EVs can be injected directly, thus appearing to be a ready-to-use drug. However, information on the required dosage of EVs is still insufficient, and comparison among experiments is difficult. EV administration dosage was mainly based on EV protein content [39–41], which may suffer from extravesicle protein contamination. Standardization of the number of EVs or of EV-donating cells could be useful.

Moreover, a relevant limit of the EV-based therapy is the absence of cell modulation by the local microenvironment encountered in vivo in the site of injury. Indeed, MSCs have been shown to modulate their therapeutic properties if exposed to a conducive inflammatory environment, so that a bidirectional influence from MSCs to the tissue and back may enhance and promote their effects [44] (Fig. 2). To mimic this aspect, MSCs could be conditioned in vitro before EV collection, using, for instance, a hypoxic treatment [45].

**MODULATION OF RESIDENT PROGENITOR CELLS**

Nephron development in mammals requires the differentiation of a renal progenitor population of mesenchymal cells into epithelial cells. Following the outgrowth of the ureteric bud, mesenchymal cells aggregate near the tips of newly formed branches, undergo mesenchymal to epithelial differentiation, and establish the renal vesicle, the precursor for the glomerular and renal tubule compartment [46]. The distal aspects of the branched duct therefore represent the niche for renal progenitors undergoing mesenchymal to epithelial transition [47]. In the adult kidney, this nephrogenic mesenchymal progenitor population disappears, possibly because of the loss of its niche [48]. Several studies, however, identified the presence of resident mesenchymal/epithelial progenitors in rodents and humans. We discuss here the possible role of these populations in renal repair and their potential therapeutic applications.

**Adult Renal Progenitors**

The presence of resident cells with the capability to differentiate into cells of the nephron and to contribute to renal repair has been shown in several murine studies [49–53]. Although these
cells were identified with different markers and were localized in different parts of the nephron, they all shared the ability to resist apoptotic damage and to proliferate during renal injury. When injected in vivo, renal stem/progenitor cells contributed to injury repair by integrating into the tubular cells and interstitial space, and when transplanted into the metanephric kidney, they integrated into the epithelial components of the nephron [49–54].

Investigating the mechanisms of proximal tubular cell regeneration, Humphreys et al. [55] showed that the repair was mediated by scattered resident injured tubular cells that had survived the injury, suggesting that regeneration should be ascribed to differentiated tubular cells undergoing dedifferentiation and therefore excluding the presence of a cell niche of stem cells with slow cycling characteristics. In contrast, experiments from Langworthy et al. [52] demonstrated a specific resident population of cells identified by the transcription factor Nfatc1. This cell population survived to apoptosis and promoted proliferation and tubule repair in a mercury chloride-induced acute kidney injury model. In particular, 75% of reconstitution of proximal tubular cells identified by the transcription factor Nfatc1 were indicated to have the characteristics after damage. Moreover, the different populations of CD133+ cells were reported in different nephron segments, including the Bowman's capsule of the glomeruli, in the proximal tubules as well as in the inner medullary papilla region, including Henle's loop and the S3 limb segment [57, 58]. CD133+ progenitors exhibited the embryonic renal markers Pax-2, Six 1, and Six 2 and several mesenchymal stem cell markers, such as CD29, CD73, and CD90 [56–58].

The exact nature of CD133+ progenitors is uncertain: they could represent a pre-existing population able to survive damage, or rather a dedifferentiated population acquiring progenitor characteristics after damage. Moreover, the different populations of CD133+ that were reported in different nephron segments exhibit different properties and possibly different functions. Cortical CD133+ cells in proximal tubules were reported to proliferate in response to renal damage [59, 60]. In fact, the number of CD133+ cells was increased in the tubules of transplanted patients undergoing delayed graft function as a result of acute renal injury [59], as well as in tubules of patients with proteinuric glomerular diseases [60].

CD133+ cells isolated from the Henle’s loop showed higher differentiation ability and stemness markers compared with those in tubules [58]. This may be due to the hypoxic environment of the inner medulla, which has been reported to induce the stem cell transcription factor OCT4A under the control of hypoxia inducible factor-1 [58]. The presence of cells with higher activation of hypoxia inducible and stem cell programs could explain the increased ability of the medulla to resist insults with respect to the cortex. In addition, these medullary CD73+/CD133+ cells were recently shown to synthesize and release erythropoietin under hypoxia [61]. Beside erythropoiesis, CD133+ cell-released erythropoietin may play tissue-specific physiologic roles such as modulation of angiogenesis and cell survival.

Experiments on the administration of CD133+ cells in murine models of glycerol-induced acute kidney injury and of adriamycin-induced glomerular damage showed improvement of the renal function, suggesting the feasibility of cell therapy with human isolated CD133+ renal progenitors [56, 57, 61, 62]. However, at present, the possible application of these cells cannot be envisaged, for several reasons such as the scarce availability of the cells, the need for compatibility among patients, and the availability of other sources of effective cells as MSCs. In any case, the possible modulation of renal progenitors in situ using pharmacological approaches, or alternatively the modulation of tubular cells toward the acquisition of progenitor characteristics to repair kidney injury, is of interest. It can also be speculated that mesenchymal-like CD133 progenitors might be directed to divergent fates, including fibrosis formation, depending on a different microenvironment or the duration of the injury [63]. The knowledge of these aspects could lead to new pharmacological approaches targeting renal progenitors. For instance, as the pharmacologic inhibition of prolyl hydroxylase was shown to enhance erythropoietin release by CD73+/CD133+ progenitors [61], a new rationale for the use of prolyl hydroxylase inhibitors in clinical settings of acute or chronic renal injury might be envisaged. In addition, the influence of currently used drugs, such as steroids and calcineurin inhibitors, on adult renal progenitors of glomeruli and tubules is of interest. For instance, angiotensin-converting enzyme inhibitors were reported to limit an excessive proliferation of glomerular progenitors in a rat model of progressive glomerular injury and to favor glomerular repair [64]. Finally, MSC therapy itself may promote progenitor cell survival and proliferation, and dedifferentiation of resident cells. The possible identification of new pharmacological therapies for progenitor cell manipulation is a promising approach for kidney regeneration.

Renal Pericytes/MSCs

Resident MSCs have been described in almost all organs as a multipotent population adjacent to endothelial cells in the microvasculature and characterized by expression of platelet-derived growth factor receptor, CD146, neuron glial antigen-2, and coexpression of other mesenchymal markers [65]. In the kidney, resident MSCs have been first isolated in the murine glomeruli [66], in the light of its vascular nature. MSCs were subsequently confirmed in the human glomeruli as CD133+/CD146+ cells with self-renewal capability, clonogenicity, and multipotency [67]. Recently, murine renal MSCs cells isolated from the adult whole kidney were shown to differentiate into rennin-producing cells [68]. The comparative evaluation of gene expression in MSCs of renal and bone marrow origin identified a selected patterns of genes in renal MSCs possibly related to a memory of tissue origin [69]. This suggests that renal MSCs might display organ-specific regenerative capacities that could overcome those of MSCs from unrelated organs and that could be exploited for therapeutic applications [69].

**ORGAN GENERATION**

Renal Organoids

Although the different cell-based experimental approaches mentioned above may represent an optimistic prospect of renal disease management, the unmet need of whole-organ renal
transplants forces us to consider other approaches of tissue engineering and whole organ culture (Fig. 1). Different strategies have been tested for constructing renal organoids from the recombination of cultured renal progenitor cells and/or primordial tissue in vitro on three-dimensional (3D) scaffolds [70–73]. A renal assist device was generated using cloned metanephros cultured on collagen-coated cylindrical polycarbonate membrane and subsequently transplanted in mice [70]. The subcutaneous implants retrieved showed functional properties, with urine-like liquid production six times higher than the control, and generated vascularized structures with tubules and glomeruli [70]. Rosines et al. [71] established in vitro a coculture system of ureteric bud and metanephric mesenchyme cells recapitulating the reciprocal induction of kidney development observed in the embryo. In the presence of growth factors, Wolffian duct was induced to bud, and each ureteric bud was induced to branch and subsequently recombined with metanephric mesenchyme. After a few days of mutual induction, cells developed a branched collecting duct system and formation of nascent nephrons resembling a late-stage embryonic kidney [71]. In a subsequent study, the authors demonstrated the feasibility to recapitulate 3D growth and branching morphogenesis in vitro when kidney rudiments were embedded in a 3D matrix [72]. Similarily, Jorakul et al. [73] developed a 3D in vitro culture system of murine primary renal cells to form tubules and glomerulus-like structures using rat tail collagen type 1 as supporting matrix. When in vitro generated kidney rudiments were implanted under the renal capsule of host rat, they vascularized, formed glomeruli [71], and survived for more than 5 weeks [72].

Recently, a new method for the development of functional organoids in the absence of 3D scaffolds was reported using single-cell suspensions of fully dissociated murine embryonic day 11.5 kidneys [74], by modification of a single cell-based dissociation-reassociation method of producing embryonic renal tissues from suspensions of embryonic mouse renal cells [75]. When this 5-day in vitro cultured structure was injected under the renal capsule of athymic rats, it developed vascularized glomeruli, mature tubular structures, and a functional engineered nephron with the capacity for filtration, reabsorption, and erythropoietin release [74]. Interestingly, human kidney epithelial cells isolated from tissues obtained from nephrectomized patients showed the ability to generate renal organoids in the absence of 3D scaffolds [76]. These cellular aggregates or spheroids could be regenerated even upon enzymatic disaggregation in vitro and expressed nephron progenitor genes such as Pax2 and Sall1. In addition, in comparison with monolayer cells, the human spheroids increased the expression of previously described renal progenitor markers, including the epithelial cell adhesion molecule, CD24 and CD133, and aldehyde dehydrogenase 1 activity [76]. However, the functional properties of these organoids were not tested.

Among the different strategies that aim to build efficient kidney tissue, the generation of renal organoids in the absence of 3D scaffolds showed interesting aspects of cell differentiation into a complex nephron-like structure, with functional properties [74, 75]. This might be due to the recapitulation of nephrogenesis mechanisms within the assay. The use of different cell types, such as induced pluripotent stem (iPS) cells, eventually differentiated in the renal lineages, could further improve this technology. However, at the moment, the use of small organoids appears quite far from the generation of the critical mass of nephrons required for renal function. Therefore, the use of decellularized kidneys engineered with different cell types, described below, appears more promising.

**Decellularized Kidney**

The kidney has a complex anatomical and physiological structure that leads to various difficulties in engineering a whole organ ex vivo. However, advances have been obtained using decellularized tissue matrices that mimic native 3D architecture to generate in vitro renal tissues in rat [77], rhesus monkey [78], and pig [79]. Kidneys were decellularized using washing detergents and seeded with different cell types, including renal fetal kidney explants and embryonic cells [77, 78]. Interestingly, decellularized matrix was reported to contain growth factors, in addition to matrix proteins [78]. More recently, a functional kidney was generated by recellularization of a rat decellularized kidney using both human endothelial cells injected through the renal artery and rat neonatal kidney cells retrogradely injected through the ureter [80]. It appears therefore that rather than stem cells, endothelial and epithelial cells, possibly of neonatal tissue, are sufficient to repopulate a kidney [80]. However, this method of generating whole organ culture is still in its early stage, and extensive research is needed to identify the optimal sample source, age, decellularization protocol, viability, and functional parameters of these cultures.

**Toward Programming: Embryonic Stem Cells and Induced Pluripotent Stem Cells**

Embryonic stem (ES) cells are pluripotent stem cells able to give rise under appropriate culture conditions to all cell lineages of the body [81]. Although research on human ES cells is limited by ethical concerns, extensive research was conducted on murine ES cells to establish methods to efficiently induce differentiation into renal cells (reviewed in [82]). In vitro, ES cells were able to generate both renal progenitors with markers of intermediate mesoderm or fully differentiated renal-like cells expressing WT-1 and rennin [83, 84]. In addition, mouse ES cells have been successfully integrated into renal structures [85–87], suggesting a possible efficacy for kidney repair. In particular, labeled ES cells microinjected into developing metanephros in organ culture were exclusively located in the cortical nephrogenic zone and differentiated into epithelial cells resembling renal tubules and, occasionally, into glomerular tufts [85–87]. Furthermore, when injected in vivo in mice, ES cells integrated into proximal tubules of newborn mice and were detectable until 7 months without teratoma formation [87].

At present, ES cell application is considered to be impractical for application in clinics because of both ethical and compatibility issues. These problems have become resolvable by using iPS cells, which can be generated from somatic cells of patients and subsequently redifferentiated/dedifferentiated in the different cell types required [88]. Because a persistent genome-wide epigenetic memory of the somatic cell of origin can be retained in iPS cells [89], the possible renal origin of iPS cells could provide some advantages for renal cell therapy or organ reconstruction. This was achieved, for instance, using iPS cells from human mesangial cells that were differentiated in podocyte and, once engrafted in a metanephric kidney, integrated in the developing glomeruli [90, 91]. iPS cells have been also obtained from renal...
tubular epithelial cells [92] and, interestingly, from human exfoliated renal epithelial cells present in the urine [93, 94]. The function of kidney lineage cells derived from ES or iPS cells remains to be determined [82].

In addition, it appears difficult to replicate in vitro the complex interactions occurring during organogenesis among cells and tissues to generate a whole organ from iPS cells. A novel possible approach for organ generation is the de novo blastocyst complementation, in which cells are microinjected into a blastocyst in which the development of a certain organ is precluded by genetic manipulation [95, 96]. Using this technique, chimeric kidneys were successfully obtained by injecting murine iPS cells into the blastocysts of anephric Sall1−/− mice [97]. In addition, the collecting ducts and the vascular system of the iPS-derived kidneys were of both donor and host recipient origin, suggesting the possibility of integrating these different cell sources [97]. The iPS-derived kidneys appeared to be functional, as shown by urine accumulation in the bladders of neonatal Sall1−/− mice derived from the complemented blastocysts. However, it was not possible to evaluate the proper function of the iPS-derived kidneys because of the lack of progression of the mice to adulthood, and further studies will be essential to dissect this point [97].

Indeed, information from clinical trials is available only for BM-MSCs. Renal progenitors, on the other hand, do not seem to represent a potential cell source for cell therapy. Understanding the molecular mechanisms involved in their proliferation and differentiation could lead to the generation of specific pharmacological approaches to enhance renal repair. It is possible, however, that renal progenitors, as well as embryonic renal cells or induced renal progenitors, could be of interest in the generation of bioengineered kidneys. Bioproducts from stem/progenitor cells, such as microvesicles and exosomes, are likely a new promising approach of reprogramming resident cells. Several issues need to be solved before a possible use of EVs can be envisaged, including the elucidation of their mechanisms of action, their biodistribution, and, in particular, their dosage. Finally, the possibility of generating renal organoids or bioengineered decellularized kidneys could represent the cure for conditions of chronic renal damage, where the endogenous mechanisms of repair cannot be stimulated for loss of renal tissue, fibrosis, and progenitor cell exhaustion. Although several cell sources are candidates for the bioengineering studies, recent studies suggest that renal differentiated cells could be sufficient to repopulate a kidney, limiting the need and related problems of stem cells.

**CONCLUSION**

In conclusion, stem/progenitor cells offer an intriguing opportunity to provide regeneration and repair of the renal tissue (Table 1). However, the high complexity of this organ and the absence of a population with the classic stem cell capability of organ homeostasis and nephron regeneration render this approach still distant. The most convincing data at the moment are the use of stem cells to enhance the intrinsic reparative capabilities of the kidney (Fig. 1). MSCs represent a promising cell resource for this approach, and the different characteristics of MSCs from different sources could be useful in defining different applications. MSCs from fetal tissues deserve a great interest for their enhanced potency, whereas those from adipose tissue present advantages of accessibility and could be ideal for autotransplants.

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**AUTHOR CONTRIBUTIONS**

S.A. and A.M.: provision of study material, manuscript writing; B.B.: conception and design, financial support, manuscript writing.

**DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST**

S.A. has compensated employment and research funding.

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