Review Article

The use of hydrogels for cell-based treatment of chronic kidney disease

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Chronic kidney disease (CKD) is a major and growing public health concern with increasing incidence and prevalence worldwide. The therapeutic potential of stem cell therapy, including mesenchymal stem cells (MSCs) and endothelial progenitor cells (EPCs) holds great promise for treatment of CKD. However, there are significant bottlenecks in the clinical translation due to the reduced number of transplanted cells and the duration of their presence at the site of tissue damage. Bioengineered hydrogels may provide a route of cell delivery to enhance treatment efficacy and optimise the targeting effectiveness while minimising any loss of cell function. In this review, we highlight the advances in stem cell therapy targeting kidney disease and discuss the emerging role of hydrogel delivery systems to fully realise the potential of adult stem cells as a regenerative therapy for CKD in humans. MSCs and EPCs mediate kidney repair through distinct paracrine effects. As a delivery system, hydrogels can prolong these paracrine effects by improving retention at the site of injury and protecting the transplanted cells from the harsh inflammatory microenvironment. We also discuss the features of a hydrogel, which may be tuned to optimise the therapeutic potential of encapsulated stem cells, including cell-adhesive epitopes, material stiffness, nanotopography, modes of gelation and degradation and the inclusion of bioactive molecules. This review concludes with a discussion of the challenges to be met for the widespread clinical use of hydrogel delivery system of stem cell therapy for CKD.

Introduction

Worldwide, the impact of chronic kidney disease (CKD) continues to rise at an alarming rate [1], and results in the progressive loss of renal function leading to end-stage renal disease (ESRD). The accumulation of interstitial collagen is a hallmark of CKD caused by inflammation, leading to the development of fibrosis.

CKD is an age-related disease [2], and is strongly associated with co-morbidities such as obesity, hypertension and diabetes mellitus [1,3,4]. An increasing aging population, coupled with the rising prevalence of metabolic syndrome, means the impact of CKD is projected to increase at epidemic proportions [3,5].

Considerable advances have been made in slowing the progression of CKD through multiple approaches (reviewed in [6]), however the only current treatments available for patients with ESRD are dialysis or kidney transplantation, which both have significant economic cost [7]. Transplantation is hampered by a shortage of kidney donors and the use of immunosuppressants to prevent rejection. Therapeutic alternatives are desperately needed to halt the progression of CKD and replace damaged cells to restore function, easing the strain on the healthcare system.

Fibrosis and loss of renal function is a common end point of most forms of progressive kidney disease [8]. Renal fibrosis is thus an excellent treatment target; however, there are limited effective antifibrotic treatments. Fibrosis, being a vital repair process, has tight regulation and redundancy, and involves multifactorial pathways that are regulated by many cell types and mediators [9-15]. As a result, therapeutic
approaches targeting a single factor or even a combination of approaches, fail to completely halt the progression of fibrosis. Hence the impetus for more sophisticated, efficacious combination therapies that are not only required to halt fibrosis, but have the capacity to induce its regression, and as such have potential to provide significant medical, social and economic benefits [9].

The therapeutic potential of stem cell therapy, including mesenchymal stem cells (MSCs) and endothelial progenitor cells (EPCs) have been widely investigated for regenerative medicine. Despite promising experimental results, there are still significant challenges before cell-based therapies can be adopted as a clinically viable option. Both MSCs and EPCs mediate their effects largely through paracrine signalling mechanisms [16-23] and require microenvironmental support to optimise cell engraftment and proliferation. However, there are significant bottlenecks in clinical translation of these promising cellular therapies due to the low rates of cell survival following transplantation that limit their therapeutic efficacy in chronic disease settings. The development of fibrosis and chronic inflammation hampers cell survival and limits the integration of the cells into the host tissue. Modulation of fibrosis and removal of the fibrotic lesion is therefore crucial to overcome these hurdles and facilitate cell integration. In addition, the low number of transplanted cells retained at the site of injury also hampers stem cell efficacy.

To overcome clinical bottlenecks, a local delivery system that increases cell survival and encourages long-term retention after transplantation could improve the efficacy and suitability of stem cell therapy in the setting of CKD. In the past decade hydrogels have emerged as a clinically viable delivery method for cell therapy, promoting retention and therapeutic agent release directly to the target organ [24-32].

In this review, we first highlight the advances in stem cell therapy targeting kidney disease, then discuss the emerging role of hydrogel delivery systems to fully realise the potential of adult stem cells as a regenerative therapy for CKD in humans, including a discussion of the features of hydrogels which must be optimised to facilitate stem cell therapy. We conclude this review by discussing further hydrogel features and barriers which are limiting the translation of hydrogel-based stem cell therapy for CKD.

Stem cell therapy for CKD
Protection and reversal of kidney damage by MSCs and EPCs in acute experimental injury models has been extensively studied and reviewed (see [33]), and the body of evidence supporting their use in CKD is growing. Adult multipotent stem cells, namely MSCs [34,35] and EPCs [23,36], minimise renal damage after acute insult and reduce the severity of fibrosis, delaying CKD. A recent systematic review of preclinical studies found that MSCs are the most well-supported cell type for treating CKD [37], that consistently improve functional and histological parameters, except glomerular filtration rate (GFR). Other cell types, including EPCs and bone marrow cells (BM) have also been effective at retarding progression of CKD, with EPCs being most effective at improving GFR [37].

MSCs
MSCs are undifferentiated multipotent adult stem cells of mesodermal origin [38], that possess attractive therapeutic qualities which can be utilised for constructing de novo tissue constructs and replacing damaged tissue [39,40]. Secondly, they can be obtained relatively easily from adult sources, plus their low immunogenicity permits allogeneic transplantation without the use of immunosuppressive drugs. Finally, they are potent trophic regulators of injured tissue microenvironment, where they modulate the inflammatory response, extracellular matrix (ECM) remodelling, angiogenesis and differentiation of tissue-specific progenitor cells [41-43]. MSCs alter the inflammatory environment via cell–cell interaction with macrophages, changing their phenotype and function to more wound-healing [16].

MSCs home to the site of injury (reviewed in [44]), then release growth factors or anti-inflammatory cytokines. By dampening inflammation, MSCs decrease the development of fibrosis and allow for vascular regeneration [21,22,43]. Some studies have observed MSC engraftment in AKI, suggesting that MSCs may have some capacity for replacing injured cells. However, the growing consensus is that MSCs mediate their effects through paracrine mechanisms and not via direct engraftment. MSC therapy has been found to be safe by meta-analysis of clinical trials, with the only associated risk being transient fever [45], although it is interesting to note that there were safety concerns in the past. One study reported MSC transformation into sarcoma cells after exogenous delivery [46], however this study did not verify the identity of the cells they delivered. While the 'MSCs' were isolated from bone marrow aspirate by tissue culture plastic adherence, the adherent culture from bone marrow was a heterogeneous population, and further isolation based on cell surface markers is required to verify a pure MSC population [47]. This highlights the importance of a stringent consensus definition of MSCs, to ensure the safety of MSC therapy.
EPCs

Endogenously recruited EPCs play a critical role in promoting neovascularisation of ischaemic tissues and induce endothelial replacement. CKD is often associated with renal hypoxia as the extensive ECM remodelling destroys the microvasculature, which promotes further fibrosis, making EPCs an attractive candidate for cell therapy. A single intrarenal infusion of autologous EPCs in experimental CKD has been shown to preserve both the renal microvasculature and existing somatic endothelial cells [36]. Delivery of EPCs has also been found to improve the function of infarcted myocardium [48] and rescue the kidney from AKI. The pro-angiogenic effects of EPCs are mediated in part through the expression of VEGF and its regulators [36,49].

Circulating EPCs are recruited to the kidney after injury which then release pro-angiogenic and pro-survival factors (for review of the mechanisms of homing, see [50]). By promoting the survival of kidney tubular cells EPCs maintain the integrity of kidney tubules, while simultaneously promoting the formation of new blood vessels, preventing further loss of tissue triggered by ischaemia. EPCs engraft into microvessels and kidney tubules where they acquire endothelial and tubular cell phenotype, respectively [51], so their mechanism of renoprotection is both direct engraftment and indirect paracrine signalling.

EPCs have been shown to be safe in numerous phase I clinical trials, and have recently been shown to maintain a stationary phase of renal function at a 1-year follow-up of CKD patients, where untreated control patients’ renal function declined [52]. For an excellent review of EPCs in CKD, see [53].

Evidence for renal repair using MSCs and EPCs

In a clinical setting, cell therapy may be considered after the onset of CKD, thus any proposed treatment strategy should be effective at improving renal function of established CKD.

MSCs and EPCs have been shown to be renoprotective in numerous injury models, both acute and chronic. While many studies have shown that infusion of stem cells at the time of injury preserves renal function, there is limited evidence confirming the rescue of function after injury is established. Table 1 summarises the studies which deliver stem cells either at the onset of injury or once damage is established using chronic injury models. The timing and cell dose of these studies are worth noting, as they vary dramatically, and timing in particular is a strong determinant of renoprotective outcome. In a recent meta-analysis of 71 articles evaluating the efficacy of cell therapy in preclinical models of CKD, it was confirmed that both MSCs and EPCs have potential to improve renal function and morphology [37].

Therapeutic delivery

Both autologous and allogeneic MSCs have potential benefits as a therapeutic approach to reduce inflammation and promote endogenous repair. Autologous cell transplantation is usually preferred over allogeneic, to decrease the risk of allosensitisation, and while some evidence suggests that the autologous delivery of MSCs derived from patients with ESRD behave typically [54,55], other evidence suggests that uremic exposure of autologous MSCs induces a procalcific phenotype and functional incompetence [56,57]. Allogeneic MSCs that are not influenced by the disease state of the patient prior to transplantation, have been shown to be safe when delivered to experimental models of kidney injury [35,58] and meta-analysis of clinical trials has shown no acute inflammatory response to unmatched MSCs [45]. Utilisation of allogeneic MSCs as ‘off-the-shelf’ products for cell therapy facilitates improved turnaround time between diagnosis and treatment as they can be rapidly propagated prior to the therapeutic administration to patients. While further investigation into the therapeutic benefit of autologous stem cells for patients with ESRD is needed, allogeneic stem cells have been shown to be safe in clinical trials.

EPCs are present in low numbers and are more difficult to isolate and culture from patients than MSCs [59]. To compound the difficulty of using autologous EPCs for CKD patients, CKD is associated with fewer circulating EPCs [60] and EPC dysfunction [61]. Another source or EPCs, such as iPSC-derived EPCs, may be a more practical solution.

A reliable and safe source of cells is a crucial determinant of the success of cell therapy. Allogeneic MSC therapy is currently the simplest, safest and most reliable approach, however it is yet to be established whether it is the most efficacious.

Secreted factors

MSC efficacy in renal repair has been largely attributed to the release of paracrine and endocrine factors [19-21], leading some groups to isolate and administer MSC exosomes and conditioned medium (CM) with promising results [18,62,63]. One notable study found that repeated intravenous delivery of embryonic stem cell derived MSC-CM reverses established experimental CKD in rats [62]. Conversely, a separate study compared the renoprotective efficacy
Table 1 Studies investigating the utility of MSC and EPC for treating CKD

<table>
<thead>
<tr>
<th>Model system</th>
<th>Cell (source, type, dose and timing)</th>
<th>Mechanism</th>
<th>Outcome</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Rodent 5/6 nephrectomy</td>
<td>Alogeneic MSCs, single or multiple iv injections of 2 × 10^6 cells, either 2 weeks after npx, or 2, 4 and 6 weeks after npx</td>
<td>Minor cell engraftment. Significant increase in anti-inflammatory mRNA expression. Multiple paracrine mechanisms including decreased EMT, fibrosis and immunomodulation</td>
<td>A single MSC infusion resulted in no improvement in renal function parameters after 4 weeks. Repeated infusions improved proteinuria and weight gain, decreased fibrosis and glomerulosclerosis, and significantly restored renal function over 8 weeks</td>
<td>[19]</td>
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<tr>
<td>Rodent 5/6 nephrectomy</td>
<td>Alogeneic MSCs, single kidney parenchymal injection of 1.5 × 10^6 cells, at the time of npx</td>
<td>Not discussed</td>
<td>Delayed progression, decreased proteinuria at day 120. No difference in histological scoring in treated or untreated, however distribution of lesions was limited with MSC treatment to the infarcted area</td>
<td>[140]</td>
</tr>
<tr>
<td>Rodent 5/6 nephrectomy</td>
<td>Alogeneic MSCs, single iv injection of 1 × 10^5 cells, 1 day after npx</td>
<td>Minor cell engraftment in the kidney. High expression of VEGF in the MSC-treated group at 1 month. Paracrine secretion of mediators.</td>
<td>Attenuates the progression of proteinuria, total protein/creatinine ratio (TP/Cr) significantly less than untreated. No significant difference in urine creatinine, urea excretion or interstitial histology during 5 months</td>
<td>[141]</td>
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<tr>
<td>Rodent 5/6 nephrectomy</td>
<td>Alogeneic MSCs, single subcapsular injection into remnant kidney of 2.5 × 10^6 cells, at the time of npx</td>
<td>MSCs were found in renal parenchyma at 5, 15 and 30 days after injection</td>
<td>Decreased hypertension, albuminuria and serum creatinine</td>
<td>[142]</td>
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<tr>
<td>Rodent 5/6 nephrectomy</td>
<td>Alogeneic EPCs, multiple iv injections of 1 × 10^6 cells, at time of npx (day 1) and day 7</td>
<td>EPCs were found in the glomeruli at 4 and 8 weeks after transfer, and in peritubular capillaries. Decreased TGF-β1 expression. Normalised the expression of inflammatory mediators, and the infiltration of inflammatory cells</td>
<td>Decreased glomerulosclerosis index, proliferation in response to tubular damage, tubulointerstitial fibrosis, blood pressure, BUN elevation, and proteinuria. Recovered body weight after 12 weeks</td>
<td>[143]</td>
</tr>
<tr>
<td>Rodent renal stenosis</td>
<td>Alogeneic MSCs, multiple iv injections of 2 × 10^5 cells, 3 and 5 weeks after left renal artery occlusion</td>
<td>Normalised the expression of inflammatory mediators and suppressed intrarenal RAS through paracrine signalling</td>
<td>Did not fully normalise SBP, but prevented further increase compared with untreated, improved structure and reduced fibrosis, improved function</td>
<td>[144]</td>
</tr>
<tr>
<td>Rodent renal stenosis</td>
<td>Alogeneic MSCs, single kidney subcapsular injection of 1 × 10^6 cells, 4 weeks after induction of RAS</td>
<td>MSC engraftment 15 days after injection, located near inflammatory cell infiltration area. Suppressed intrarenal RAS. Prevented EMT through down-regulation of TGF-β. Induced proliferation</td>
<td>Reduced SBP. Maintained Na⁺–K⁺-ATPase activity and expression. Significantly improved renal morphology, reduced fibrosis, and improved renal function</td>
<td>[145]</td>
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<tr>
<td>Rodent Alport disease</td>
<td>Alogeneic MSCs, multiple iv injections of 1 × 10^6 cells at 6, 7, 8 and 9 weeks of age (renal damage is established)</td>
<td>Low MSC engraftment. Did not affect numbers of Mac2-positive macrophages. Increased VEGF expression in MSC-treated cohort may have prevented loss of peritubular vasculature</td>
<td>Reduce interstitial fibrosis and improved microvasculature but does not restore renal function or improve survival</td>
<td>[146]</td>
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<tr>
<td>Rodent anti-Thy1.1 nephritis</td>
<td>Alogeneic MSCs, single intrarenal injection of 2 × 10^6 cells, 2 days after disease induction</td>
<td>MSC engraftment after 8 days, localised to glomeruli. Likely renoprotection via paracrine effects</td>
<td>Preserved renal function, some maldifferentiation into adipocytes. MSC significantly reduced serum creatinine, proteinuria, loss of glomeruli and interstitial fibrosis</td>
<td>[147]</td>
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<tr>
<td>Rodent streptozotocin-induced diabetes</td>
<td>Alogeneic MSCs, single iv injection of 0.5 × 10^6 cells, 25 days after first STZ dose</td>
<td>Regenerate β-pancreatic islets</td>
<td>Glucose regulation over 2 months. Prevented renal damage for at least 2 months</td>
<td>[148]</td>
</tr>
<tr>
<td>Porcine renal stenosis</td>
<td>Autologous EPCs, single intrarenal injection of 10 × 10^6 cells, 6 weeks after induction of RAS</td>
<td>Promotion of angiogenesis and the maturation of new vessels. Autocrine and paracrine factors likely key for improvement. Cell engraftment after 4 weeks. Direct engraftment and differentiation, and recruitment of circulating or resident stem cells may play a role</td>
<td>Renal blood flow, microvasculature, GFR and fibrosis significantly improved</td>
<td>[36]</td>
</tr>
<tr>
<td>Porcine renal stenosis</td>
<td>Autologous EPCs or allogeneic MSCs, single intrarenal injection of 10 × 10^6 cells, 6 weeks after induction of RAS</td>
<td>EPCs preserved microvasculature, MSCs were immunomodulatory and antifibrotic</td>
<td>Similarly decrease kidney injury by different mechanisms. MSCs elicited slightly superior improvement of renal function. GFR significantly decreased with EPC treatment, further improved with MSC. RBF similarly improved</td>
<td>[66]</td>
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of MSCs with MSC-CM and found that while MSCs confer significant protection from IR injury, intravenous delivery of MSC-CM did not preserve renal function, even when high doses were administered daily for 7 days. This supports the notion that renoprotection afforded following MSC delivery is due to paracrine, rather than endocrine factors [64]. Another study found that MSC-CMs were more efficacious at reducing fibrosis in a unilateral ureteral obstruction model than MSCs [65].

As previously discussed, the mechanisms of renoprotection by EPCs are both direct engraftment and indirect paracrine signalling, stimulating resident cells to repair the injury. EPCs promote angiogenesis by releasing various cytokines, including VEGF, insulin-like growth factor (IGF) 1 (IGF-1) and stromal cell-derived factor 1 (SDF-1). Interestingly, a comparison of the mechanism of renoprotection by EPC and EPC-CM found that endoplasmic reticulum stress and apoptosis in porcine kidney tubular cells was inhibited by EPCs but not EPC-CM, suggesting that some mechanisms of EPC renoprotection rely on cell–cell contact [66].

The advantages of using stem cells over CM or exosomes are not conclusively defined; for a systematic review of preclinical studies using this approach, see [17].

Challenges
A major challenge in the systemic delivery of transplanted cells is targeting treatment to the desired organ. While it has been shown that systemically delivered MSCs and EPCs home to the injured kidney, only a small fraction of the administered cells reaches the target tissue. A large proportion of cells become trapped in the lungs before reaching the target organ [67], leading investigators to administer high doses. This can be technically challenging and introduces risk of pulmonary embolism. Delivering cells locally to the target organ may circumvent this issue, and pre-clinical

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<tbody>
<tr>
<td>Rhesus monkey cisplatin nephrotoxicity</td>
<td>Autologous MSCs, single intrarenal injection of 5 × 10⁶ MSC/kg, Either 4 days (preventative) or 6 months (stable) after injury</td>
<td>Improvement of renal pathology in preventative model may facilitate protection of renal function, whereas in the stable model the kidney sustained extensive structural damage</td>
<td>MSCs improve renal function and slow fibrosis in the preventative model. No effect on fibrosis or renal function on the stable model</td>
<td>[149]</td>
</tr>
<tr>
<td>Human CKD, Phase I clinical trial</td>
<td>Autologous CD34+ (EPCs), single intrarenal injection of 5 × 10⁶ cells. After 4 consecutive days of G-CSF treatment, stage III or IV CKD established</td>
<td>EPCs might play a role in endothelial cell repair and angiogenesis/neovascularisation</td>
<td>100% safe, 100% 1-year survival rate. Reduce/halt decline of renal function over 1 year</td>
<td>[52]</td>
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<td>Human atherosclerotic renovascular disease (RVD), Phase 1/2A clinical trial</td>
<td>Autologous MSCs, single intrarenal injection of 1 × 10⁶ or 2.5 × 10⁵ cells/kg, RVD established</td>
<td>Kidney hypoxia decreased in MSC-treated kidneys. Renal vein levels of VEGF decreased after MSC treatment, as did inflammatory cytokines</td>
<td>No adverse reactions. Preserved cortical blood flow and improved oxygenation better than standard medication alone for 3 months</td>
<td>[150]</td>
</tr>
<tr>
<td>Human diabetic nephropathy, NCT01843387</td>
<td>Allogeneic mesenchymal precursor cells (rexlemestrocel-L), single iv injection of 150 × 10⁶ or 300 × 10⁶ cells</td>
<td>Not discussed</td>
<td>Safe and well tolerated. Trend to stabilise or improve eGFR in 150 × 10⁶ cells group</td>
<td>[151]</td>
</tr>
<tr>
<td>Human ADPKD, Phase 1 clinical trial NCT02166489</td>
<td>Autologous MSCs, single iv injection of 1 × 10⁶ cells/kg</td>
<td>Not discussed</td>
<td>Safe and well tolerated. No significant change in eGFR, serum creatinine or kidney morphology</td>
<td>[152]</td>
</tr>
<tr>
<td>Human refractory SLE NCT00698191</td>
<td>Allogeneic MSCs, single iv injection of 1 × 10⁶ cells/kg</td>
<td>Not discussed</td>
<td>Safe and well tolerated. Decrease in SLEDAI score and 24-h proteinuria</td>
<td>[153]</td>
</tr>
<tr>
<td>Human refractory SLE NCT01741857</td>
<td>UC-MSC, two iv injections of 1 × 10⁶ cells/kg, 7 days apart</td>
<td>Not discussed</td>
<td>Safe and well tolerated. 32% patients achieved major clinical response, 28% achieved partial clinical response, 12% and 16% patients relapsed at 8 and 12 months, respectively. Decreased proteinuria, albuminuria and serum creatinine</td>
<td>[154]</td>
</tr>
<tr>
<td>Human lupus nephritis NCT01539902</td>
<td>UC-MSCs, two iv injections of 2 × 10⁶ cells/kg, 7 days apart</td>
<td>Not discussed</td>
<td>Safe and well tolerated. No difference in achievement of remission, trial was abandoned</td>
<td>[155]</td>
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Abbreviations: BUN, blood urea nitrogen; eGFR, estimated GFR; EMT, epithelial to mesenchymal transition; iv, intravenous; G-CSF, granulocyte-colony stimulating factor; Npx, nephrectomy; RAS, renin-angiotensin system; RBF, renal blood flow; SBP, systolic blood pressure; SLE, systemic lupus erythematosus; TGF-b, transforming growth factor-beta; VEGF, vascular endothelial growth factor;
Figure 1. Diagrammatic summary of the relationship between kidney injury and repair and hydrogel-assisted cell therapy

Hydrogels can support (A) MSCs and (B) EPCs to repair/prevent CKD. Kidney damage (1) (such as toxic insult, hypertension or hypoxia) results in damaged cells, resident immune cells and infiltrating immune cells releasing inflammatory mediators (2). These mediators are detected by the transplanted cells in hydrogel, which respond by releasing paracrine factors (3). MSCs (A) release anti-inflammatory, pro-survival and pro-regenerative factors, including VEGF, HGF and IGF-1, and regulate the expression of TGF-β, TNFα, IL-4, -6 and -10, among others (4). EPCs (B) recruit endogenous endothelial progenitors and promote angiogenesis, resulting in the preservation of microvasculature and prevention of tubular atrophy (4). These mechanisms may prevent further insult or repair the damage accumulated in CKD (5).

studies have shown that local delivery has a similar efficacy to systemic delivery [68]. However, irrespective of delivery mode cells are only transiently localised to tissues following transplantation before being cleared from the body.

As previously described, the factors secreted by MSCs and EPCs have been found to promote renal repair when administered as CM or exosomes, and in this way stem cells can be considered a ‘living drug delivery system’ [69]. The therapeutic effectiveness of stem cells delivered systemically is limited due to their transient survival, which may not be suitable to treat chronic conditions. Conversely, a local depot of stem cells which are protected from the immune system may enable them to reduce kidney inflammation over longer periods. Long-term retention of stem cells by, for example hydrogel encapsulation may reduce the progression of CKD by the release of secreted factors in response to local inflammation, ensuring that inflammation is transient by promoting an anti-inflammatory environment, as depicted in Figure 1.

Hydrogel delivery systems

Hydrogels may offer a solution to the current challenges faced by the translation of cell therapy for patients with CKD. Hydrogels are porous networks that exhibit tissue-like water content and elastic properties. Their applications are far-reaching, including delivery of cells and other therapeutics such as miRNA and peptides to tissue-engineering scaffolds, cell culture substrates to biomedical devices.

Hydrogels can enhance cell therapy in numerous ways. Firstly, they can be utilised for local delivery of stem cells and bioactive molecules, minimising systemic side effects and maximising the proportion of cells reaching the desired site (for review, see [70]). Many hydrogels are injectable, thus providing a relatively non-invasive method for local delivery into the target area [71] facilitating precise dosage of stem cells. Secondly, hydrogels protect cells from shear-stress injury during syringe injection, preserving the quality of transplanted cells [72]. Thirdly, they can protect stem cells from hostile microenvironments including local inflammation, ECM remodelling or necrosis [73,74], which increases cell viability and the capacity for bioactive molecule release [75]. Hydrogels which are decorated with bioactive molecules can promote tissue repair [76-79], and the prospect of a combination therapy involving local stem cell delivery, a cell-supporting hydrogel scaffold and targeted bioactive molecule release may pave the way for a new era of regenerative medicine.
Hydrogels used for cell and drug delivery

Injectable hydrogels have been utilised to promote tissue regeneration experimentally in numerous tissues, including skeletal bone and cartilage [80-82], vocal fold [28], central and peripheral nervous system [31,32,83-85], adipose tissue [86], cardiac tissue [25-27] and kidneys [73,76,77,79,87-90].

Hydrogels designed for the therapeutic delivery of encapsulated stem cells must optimise certain biological and mechanical properties, as discussed in the following sections and Figure 2.

Biocompatibility

The combined effects of each of the following hydrogel features determine the overall biocompatibility. Biocompatibility describes the interaction between the hydrogel material, the encapsulated cells and the surrounding tissue. The hydrogel should elicit minimal inflammatory response, as this triggers the foreign body response, leading to a fibrotic capsule around the gel, hampering cell survival and limiting bioactive molecule efflux [91]. It must support the maintenance, proliferation or differentiation of stem cells, as appropriate for the application. This can be achieved through the inclusion of ECM protein epitopes, such as RGD (from fibronectin) or SKAV (from laminin), or by using cell-adhesive molecules as the building blocks for the gel, such as collagen (review [92]), hyaluronic acid (HA) [76,77,87,93], chitosan [79,89] or gelatin [78,94].

Stiffness and nanotopography

With regards to stiffness, hydrogels should have the appropriate mechanical properties for the target tissue, for instance a load-bearing tissue such as muscle requires a hydrogel that can withstand greater forces than one designed for brain tissue. Generally, matching the hydrogel stiffness to the tissue stiffness protects encapsulated cells and the hydrogel itself from mechanical stress and strain.

Studies in the emerging field of mechanobiology demonstrate that the stiffness and nanotopography of the 3D microenvironment play an important role in cell fate determination, proliferation, migration and cell survival [95-100]. For this reason, it is vital to consider the physical microenvironment the hydrogel provides to encapsulated cells. For instance, a hydrogel which promotes an osteogenic phenotype would be inappropriate for adipose tissue engineering. For CKD, we suggest that the hydrogel should maintain MSCs and EPCs in their naive state to take advantage of their anti-inflammatory, antifibrotic and pro-angiogenic paracrine signalling.

Gelation and injectability

Rapid gelation ensures homogeneous distribution of cells within the hydrogel, which promotes cell growth and an even release of paracrine signalling molecules. The rate and mechanism of gelation are of paramount importance when considering the clinical application of any hydrogel. For example, rapid gelation of a hydrogel delivered by catheter may block the tubes or the biological components (such as cells) may be damaged by exposure to un-polymerised hydrogel precursors or the process of polymerisation, particularly if UV light or chemical cross-linking is required.

Injectable hydrogels are either liquid upon delivery and polymerise in situ or are shear thinning, such that application of shear force through a needle triggers a gel–sol transition, which rapidly reverses when shear force ceases.

There are numerous mechanisms for in situ gelation, including self-assembly, thermal and pH triggers, photopolymerisation or covalent cross-linking. Of these mechanisms, self-assembly and thermal triggers pose the least risk to surrounding tissue and encapsulated cells. Large variation in pH, exposure to UV light or forming covalent cross-links using chemicals can be deleterious due to the high energy. Visible-light photopolymerisation does not carry the risk of UV, but this class of materials is still in its infancy.

Bioactive molecule delivery

Hydrogels can include therapeutic agents, and local delivery could enable drugs with poor oral availability, or adverse side effects with systemic delivery, to become clinically viable. A number of groups have incorporated growth factors and small molecules into their hydrogel [79,94]. For example, Burdick et al. developed HA with IL-10 covalently bound, utilising the anti-inflammatory properties of IL-10 [76,77]. MSCs are known to modulate IL-10, and it has been suggested that it is one of the anti-inflammatory mechanisms of renoprotection [101]. Therapeutic agents may be simply encapsulated in the hydrogel by mixing before gelation, allowing them to escape by diffusion. This would promote a bolus release profile, quickly delivering the entire payload. Covalent binding or cleavable linkers can promote a slower release profile, extending the duration of treatment. For review, see [102].
Figure 2. Choosing hydrogel features to maximise biocompatibility and therapeutic efficacy

(A) Gelation can be broadly categorised as self-assembly or chemical polymerisation. Self-assembly is safer for encapsulated cells and surrounding tissue, but chemical polymerisation tends to result in more robust hydrogels. Rapid gelation ensures homogeneous cell distribution, which enhances cell proliferation and bioactive molecule release. Slower gelation allows cells to sink and collect at the bottom, resulting in less proliferation. (B) Degradation can occur through enzymatic degradation, hydrolysis, mechanical degradation or triggered disassembly depending on the material, the gelation method and the environment. Once degraded, the hydrogel can release encapsulated cells which migrate to the injured tissue. Non-degrading hydrogels can retain stem cells, protecting them from the injured microenvironment while they release paracrine factors. The degradation can be tuned to release cells at a desired rate. (C) Material stiffness can direct cell behaviour, including differentiation, proliferation, apoptosis, migration and cell secretome. (D) Bioactive molecules can be delivered using hydrogels. Simply mixing the substance into the hydrogel results in encapsulation, which is released as a bolus. Tethering the substance to the hydrogel fibers, with cleavable or non-cleavable linkers, can result in sustained, slow release. (E) Fibre density can influence cell behaviour, as it places limits on cell spreading and organisation which promotes different phenotypes. The type, density and availability of cell-adhesive epitopes can also promote specific phenotypes.
Degradation
Degradation rate and products must be taken into consideration for any biomedical device. Degradation products may not be biocompatible, hence long-term studies are essential to validate material safety over the lifetime of the product. Slow degradation is advantageous in tissue engineering when the goal is to facilitate sustained bioactive molecule release or cellular integration into a scaffold, since re-population of a scaffold may take many months [103,104]. Rapid degradation may be advantageous when bolus delivery of drugs or cells is desired, or when the hydrogel may obstruct a tubule or vessel. For the treatment of CKD, we propose that a long-term (slow or non-degrading) hydrogel is ideal, that can continuously release antifibrotic signals to the kidney when cells are stimulated by inflammation in surrounding tissue, via long-term retention of stem cells in an undifferentiated state. This is so that stem cell therapy can be an effective treatment irrespective of underlying pathology, by treating the fibrosis which is the common result of CKDs.

Preclinical trials
At present there are very few studies utilising hydrogels for cell therapy in CKD, however hydrogel-based stem cell therapies have restored function in a range of other models of chronic disease, including myocardial infarction [105-113], diabetes [114-116] and diabetic wounds [117-120], stroke [121-126], spinal cord injury [127] and osteoporosis [128]. The application of hydrogels in CKD is underdeveloped, but the great strides being made in other chronic diseases is promising for new hydrogel materials to catapult this growing field forward.

Hydrogel-based stem-cell therapies have been extensively studied for myocardial infarction. Similar to studies in kidney, the vast majority of research has been conducted in acute models, where the treatment has been administered either at the time or very shortly after the injury. However, there have been studies where the treatment was administered 1–4 weeks after injury; once the scarring is established. Overall, while treatment of the established disease is less effective than treatment at the time of injury, these studies report histological, conductive [106] and functional improvement [105,109-112]. For comprehensive reviews of hydrogel-based stem cell therapies for the heart, refer to [107,108,113].

Hydrogels have been used to improve cell-based therapies for diabetes, specifically pancreatic islet transplantation [114-116] which utilises the slow degradation and high biocompatibility of alginate hydrogels in combination with MSCs to improve the survival of pancreatic islets, and dressings for diabetic wounds [117-120] which are hydrogels laden with MSCs or EPCs to promote a wound-healing inflammatory profile.

Few hydrogel materials have been developed to aid stem cell therapy for kidney diseases. The following section and Table 2 summarises hydrogels developed to date for this purpose. There is no single optimal hydrogel that has the capacity to reverse CKD. As such, each of these materials, and the many new materials being created, must balance the previously discussed features to maximise their therapeutic efficacy.

HA
HA has been used clinically for over 30 years, and when chemically modified it can form biodegradable, cell-supporting, viscoelastic hydrogels which can be utilised for drug delivery or stem cell therapy. A number of studies have shown that HA-based hydrogels enhance stem cell therapy [73,74,90,129].

Delivery of HA-embedded eEPCs improved renal function of mice with either adriamycin-induced nephropathy or ischaemia–reperfusion injury, whether it was transplanted in the kidney or the ear [74]. The hydrogel was prepared using a commercially available kit, which includes thiol-modified HA and thiol-modified collagen, cross-linked with a thiol-reactive polyethylene glycol diacrylate (PEGDA) cross-linker. Pronectin and stromal cell-derived factor (SDF)-1 were also incorporated into the hydrogel to improve cell viability. EPCs homed injured kidney after the hydrogel was enzymatically degraded using hyaluronidase and collagenase, where they reduce cell death and fibrosis, and preserve renal function. EPCs embedded in hydrogel were more beneficial than systemically delivered EPCs [90].

Transplantation of mouse eEPCs embedded in a HA-PEGDA hydrogel protected the cells and kidneys from LPS-induced endotoxaemia [73]. These hydrogels were prepared as per the previous example, without the inclusion of SDF-1. eEPCs protected the kidneys from fibrosis and ameliorated renal haemodynamics. Greater preservation of renal function was observed after hydrogel-embedded EPC treatment compared with IV eEPC delivery. Interestingly, creatinine levels were most improved when encapsulated EPCs were injected into the ear, however subcapsular delivery was more effective overall at maintaining renal function, possibly due to greater cell retention in the hydrogel-treated kidneys.
<table>
<thead>
<tr>
<th>Material</th>
<th>Hydrogel</th>
<th>Injury type</th>
<th>Cell type, number</th>
<th>Delivery</th>
<th>Outcome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>HA</td>
<td>HyStem Hydrogel Kit (BioTime)—HA and collagen cross-linked with PEGDA plus Pronectin and SDF-1</td>
<td>Rodent Adriamycin nephrotoxicity or bilateral ischaemia</td>
<td>Allogeneic eEPCs, 5 × 10^5 cells</td>
<td>Either 10 μl to subcapsular space (both sides), 10 μl to ear (both sides) or single iv injection</td>
<td>Cells retained non-differentiated phenotype in hydrogel. Hydrogel protect EPCs from cytotoxic insult. Treatment preserved renal function 36 h after either ischaemia or adriamycin insult. Blood flow was fully rescued after femoral artery ligation, indicating angiogenesis. Hydrogel increased cell engraftment in kidneys</td>
<td>[74]</td>
</tr>
<tr>
<td>HA</td>
<td>HyStem Hydrogel Kit (BioTime)</td>
<td>Rodent LPS-induced endotoxemia and CLP-induced sepsis</td>
<td>Allogeneic eEPCs, 5 × 10^5 cells</td>
<td>Either 10 μl to subcapsular space (both sides), 10 μl to ear (both sides) or single iv injection</td>
<td>Hydrogel-embedded EPCs preserved renal haemodynamics and function better than IV EPCs. Fibrosis was decreased 2 months after delivery. Cytokine profile indicates reduced inflammation. Hydrogel increased cell engraftment in kidneys</td>
<td>[73]</td>
</tr>
<tr>
<td>HA</td>
<td>HyStem Hydrogel Kit (BioTime)</td>
<td>Rodent LPS-induced endotoxemia</td>
<td>Allogeneic MSCs (5 × 10^5) and EPCs (5 × 10^5), total 1 × 10^6 cells</td>
<td>Either 10 μl to ear (both sides) or single iv injection</td>
<td>Hydrogel protects EPCs and MSCs from LPS, viability improved when co-embedded compared with single cell type. Hydrogel-co-embedded EPCs and MSCs preserved renal haemodynamics and renal function better than IV EPCs or MSCs. Co-delivered EPCs and MSCs preserved M2 macrophage phenotype, as reflected in cytokine profile</td>
<td>[129]</td>
</tr>
<tr>
<td>Chitosan</td>
<td>CS-IGF-1C (1C domain of IGF grafted on to CS by click reaction)</td>
<td>AKI</td>
<td>Allogeneic MSCs, 1 × 10^6 cells</td>
<td>Left kidney cortex, three sites, 30 μl total</td>
<td>Hydrogel improved cell retention, enhanced MSC anti-inflammatory and pro-angiogenic effects. Co-transplantation of MSC with hydrogel enhanced MSC renoprotection, improving renal function and decreasing renal fibrosis</td>
<td>[89]</td>
</tr>
<tr>
<td>Collagen</td>
<td>Thermally gelling collagen/poly(γ-glutamic acid) loaded with α-lipoic acid (LA)</td>
<td>Rodent cisplatin-induced renal dysfunction</td>
<td>Allogeneic MSCs, 1 × 10^6 cells</td>
<td>Kidney cortex, 20 μl</td>
<td>Hydrogel improved MSC retention. MSC and LA had a combinatorial protective effect on renal function and histology</td>
<td>[139]</td>
</tr>
<tr>
<td>Collagen</td>
<td>Decellularised bovine pericardium</td>
<td>Rodent 2/3 and 5/6 nephrectomy</td>
<td>Allogeneic MSCs, cell number unclear</td>
<td>Renal capsule removed, biomaterial sutured adjacent to renal surface</td>
<td>Biomaterial treatment was effective in the 2/3 nephrectomy model in preserving renal function and histology. The effect of treatment was still positive but less dramatic in the 5/6 model. Treatment decreased monocyte/macrophage infiltration in both models</td>
<td>[156]</td>
</tr>
</tbody>
</table>

Abbreviations: CLP, cecal ligation puncture; CS, chitosan; eEPC, embryonic-derived endothelial progenitor cell; iv, intravenous; LPS, lipopolysaccharide; PEGDA, polyethylene glycol diacylate.

The subcutaneous transplantation of HA-embedded MSC and EPC co-culture preserved renal function after LPS insult [129]. This study also used the same hydrogel kit as the previous two examples. The hydrogel protected the transplanted cells from LPS, conferring more renoprotection than systemically delivered MSCs and EPCs [129]. The authors suggest that part of the renoprotection was due to MSCs and EPCs priming macrophages to an anti-inflammatory wound-healing phenotype and function [129]. Macrophage polarisation in CKD plays an important role in the progression of fibrosis and loss of renal function, dependent on the polarisation to either M1 or M2, or inflammatory or wound healing phenotypes, respectively. For further discussion of the roles of macrophages in CKD, see the following reviews [130-132].
While HA hydrogels are a promising tool for cell therapy, HA is rapidly degraded in the injured kidney due to high expression of hyaluronidases, limiting the duration of retention [133].

**Chitosan**

Chitosan, a polysaccharide derived from chitin, has been widely used as a biomaterial due to its biocompatibility, biodegradability to non-toxic products and wound-healing properties. The degree of cross-linking between chitosan molecules determines the porosity and stiffness of the hydrogel. Numerous studies utilise chitosan for stem cell delivery, particularly for cartilage and bone regenerations (for recent reviews, see [134,135]).

Injection of thermosensitive chitosan chloride hydrogel with adipose-derived MSCs into the kidney cortex preserved renal function after AKI [136]. Cell retention, survival and proliferation were improved compared with injection in PBS, which suggests that retention is an important determinant of efficacy [136]. At 4 weeks, significant improvement in renal function and kidney tubular structure suggests that a thermally triggered chitosan hydrogel is a potential delivery system for MSCs in AKI.

Recently, Feng et al. [89] created a chitosan-based hydrogel which incorporates the C-domain of IGF-1 (CS-IGF-1C), a pro-mitogenic growth factor. Co-transplantation of CS-IGF-1C hydrogel with adipose-derived MSCs in ischaemic kidneys ameliorated renal function by promotion of stem cell survival, angiogenesis and attenuation of fibrosis [89]. The hydrogel promoted increased cell retention, and no ectopic differentiation was observed. The authors posit that improving recovery after AKI reduces risk of patients developing CKD, however this strategy is yet to be trialled in an established CKD model.

**Collagen**

Collagen is a long fibrillar protein which is a major structural component of the ECM. Since collagen is part of the native ECM, it is inherently bio compatible and cell-supporting. It is also biodegradable, although this can be chemically modulated. However, as it is a protein, collagen can be difficult to sterilise without altering the chemical structure. Collagen hydrogels can be made in numerous ways, the simplest of which is by decellularising native collagen-rich tissues, such as the pericardium, leaving a collagen scaffold which can be used as a biomaterial. Collagen can also be isolated from different tissues, such as rat tails, and converted into a powder which can be chemically modified to produce different hydrogel properties. For comprehensive reviews of collagen hydrogels, see [92,137,138].

Injection of a collagen/poly(γ-glutamic acid) hydrogel loaded with MSCs and the antioxidant drug \(\alpha\)-lipoic acid protects from cisplatin-induced renal dysfunction in mice [139]. At room temperature the hydrogel is fluid, once injected gelation is thermally triggered at 37°C, which makes the handling and delivery more straightforward than previous collagen-based hydrogels. This hydrogel system promotes greater cell retention at the delivery site, and releases \(\alpha\)-lipoic acid more slowly than injection alone.

**Future perspectives**

The economic burden of CKD is staggering; thus, the overarching goal is to decrease dependence on renal replacement therapies. Hydrogel delivery systems for stem cell therapy hold great promise as a new treatment strategy for treating kidney disease. In the long term it may be feasible to give patients with kidney disease or associated co-morbidities, such as obesity, hypertension and diabetes, hydrogel-based stem cell therapy to slow, halt or reverse progressive kidney damage, easing the demand for renal replacement therapies.

The naturally derived hydrogels discussed in this review demonstrate strengths and shortcomings of hydrogel-based stem cell therapy. What is currently required are materials which are more easily modified, multifunctional and longer lasting to fine-tune this therapeutic approach.

To conclude this review, we highlight the following areas for which ongoing research will potentially yield exciting advancements in the application of hydrogels for stem cell therapy, in the case of CKD and other chronic fibrotic diseases.

At its core, the key to create a hydrogel is designing an artificial microenvironment for a specific outcome. For CKD stem cell therapy, the aim is to maximise stem cell efficacy in repairing damaged kidneys, noting that different microenvironments will bring different reparative mechanisms to the fore. A combination of antifibrotic, pro-angiogenic, pro-regenerative and anti-inflammatory mediators would likely be ideal, thus the influence of the hydrogel on these behaviours must be carefully considered. Researchers must question whether the constructed microenvironment should mimic healthy kidney tissue or a stem cell niche, or if an alternative approach would be more
beneficial. Which set of signals best directs encapsulated stem cells to repair the kidney? The range of microenvironments which can be constructed with our current technologies are staggering, therefore a balance must be uncovered between creating the perfect hydrogel and developing an effective therapy.

Another important hydrogel feature to consider is whether the niche should be degraded, releasing the transplanted cells or resist degradation and have greater retention. Would cells be more effective as ‘living drug delivery’ units which remain locked in the hydrogel and release molecules that diffuse to the target? From a clinical perspective, the trade-offs between therapeutic efficacy and invasiveness of delivery are critical to consider. MSCs and EPCs respond to the damaged microenvironment by releasing a suite of reparative factors, so it stands to reason that the cells need to be able to detect the local microenvironment, and subsequently respond. If the cells were immobilised in a hydrogel, they must be in close proximity to the kidney to be effective. If the cells were to escape the hydrogel, more distant implantation sites become viable, including subcutaneous injection. Experimental studies tend to show that for CKD the retention of stem cells is a critical determinant of efficacy, however more studies are required to understand the benefits of degrading compared with non-degrading hydrogels, and how this should be translated into the clinic.

An important property which was largely overlooked until recently is the stiffness of the hydrogel material. Stiffness can direct lineage determination, so it is important to consider whether the goal is to promote or prevent stem cell differentiation. This brings us back to a previous point, whether to mimic the kidney or the stem cell niche to guide the design.

The question of when to deliver the treatment, while critical to clinicians, has been very poorly characterised in experimental studies. Few studies, and none using hydrogels, compare stem cell-treated mice at different stages of CKD progression. Most provide treatment at the time of injury, and while this is an important first step, it is not relevant to treatment in the clinic. Patients with kidney disease typically do not present until the end stage, so a challenge for researchers in this space is to show that their therapy has positive outcomes in advanced CKD models.

In summary, hydrogel-based stem cell therapy remains a promising therapeutic approach to reduce or reverse progressive kidney damage and experimental studies indicate that it is more effective than intravenous stem cell delivery. The current advances seen in hydrogel-based materials suggest that a longer lasting, more effective treatment for CKD may soon be realised.

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Competing interests
The authors declare that there are no competing interests associated with the manuscript.

Abbreviations
AKI, acute kidney injury; CKD, chronic kidney disease; CM, conditioned medium; ECM, extracellular matrix; EPC, endothelial progenitor cell; ESRD, end-stage renal disease; GFR, glomerular filtration rate; HA, hyaluronic acid; IGF, insulin-like growth factor; IPSC, induced pluripotent stem cells; IR, ischemia reperfusion; MSC, mesenchymal stem cell; PEGDA, polyethylene glycol diacrylate; SDF-1, stromal cell-derived factor 1; UUO, unilateral ureteral obstruction; VEGF, vascular endothelial growth factor.

References


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