BMC Medicine

REVIEW

Open Access

Cell therapy strategies for organ regeneration

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Abstract

The development of techniques to culture and differentiate adult and pluripotent stem cells into diverse cell types over the past decades has sparked an increasing interest in the use of cells for organ regeneration. Such therapies aim to replace lost or damaged cells with functional ones. This can be achieved either through tissue engraftment of therapeutic cells or via their paracrine effects on resident cells, thereby offering a potential cure for debilitating degenerative diseases. The development of regenerative cell therapies, however, is ultimately complex. Effective cell therapy requires the delivery and successful engraftment of therapeutic cells to the correct location or sufficient paracrine activity, while ensuring safety is key to gaining support from funders, caregivers, and patients. A wide variety of cell sources has been used for the development of regenerative cell therapies, ranging from mesenchymal stromal cells (MSC) that act to stimulate local progenitor cells through their secretome to tissue-specific cell types differentiated from adult or pluripotent stem cells and organoids that engraft in tissues. While cell administration to patients is challenging based on both practical and ethical perspectives, the development of techniques to preserve transplant organs ex situ on machine perfusion devices offers a unique opportunity for studying regenerative cell therapy for organ repair in a safe and controllable environment. The present review addresses the current progress of cell therapy for organ regeneration of the intestine, kidney, liver, lung, and heart and discusses the challenges and opportunities of this potentially curing therapeutic approach.

Keywords Cell therapy, Engraftment, Organ transplantation, Regeneration, Stem cells

Background

The fundamental goal of medicine to improve patients' health has been the same throughout the ages. However, the available tools and knowledge have advanced significantly over time. Nonetheless, both traditional and modern therapies, whether herbal or pharmacological, primarily focus on alleviating disease symptoms or delaying disease progression. Many diseases we encounter have a degenerative nature, resulting in the loss of functional cells, often accompanied by an increase in nonfunctional cells and fibrosis. To reverse the impact of such degenerative diseases, regenerative therapies provide a promise.

Regenerative therapy seeks to restore the normal function of cells or replace lost or nonfunctional cells to repair organ or tissue function. This can be achieved through drugs that trigger the remaining endogenous progenitor cells. However, in many cases, these cells are malfunctioning or present in insufficient numbers. In such cases, regenerative therapy relies on cell replacement. The identification of diverse types of adult stem cells from the 1960s onwards [1–5] and human embryonic stem cells

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in 1998 [6], the technology to generate induced pluripotent stem cells from somatic cells in 2007 [7], and the development of protocols to differentiate stem cells into diverse types of functional cells and organoids 2009 [8, 9] have offered the necessary tools for the development of regenerative cellular therapies.

The ability to expand and differentiate cells in vitro in combination with advanced gene editing techniques has made it possible to study human development in vitro, model diseases, and examine the toxicity and effects of (pro)drugs. It has also led to the development of successful cell-based therapies, particularly in the fields of hematology and oncology, where chimeric antigen receptor T cells (CAR T cells) are used to destruct hematological tumors. Today, over 34,000 patients have been treated with commercially available CAR T cells [10]. Compared to the success of CAR T-cell therapy, cell therapy for organ regeneration is still in its infancy.

The field is searching for solutions to efficiently deliver cells with regenerative capacity to the right location. While stimulation of resident progenitor cells via the secretome of administered cells or via immune cell intermediates can be effective as a regenerative therapy, replacement of lost or malfunctioning cells through the engraftment of therapeutic cells offers a more controlled and possibly long-term manner of regeneration. Depending on the morphology of the target organ, there are different routes for delivering cells to their intended location (Fig. 1). The intestine has been a prime target for cell replacement studies, partly due to ease of access through the intestinal lumen, which allows for cell delivery right at the injury site. Other organs may be accessed via the vascular system. Besides being invasive, administration via the vascular system also carries the risk of cells escaping the target organ and accumulating at off-target locations. A technological advancement that can aid in the delivery and monitoring of regenerative cells is ex situ machine perfusion of transplant organs [11]. Machine perfusion of organs, such as the liver, kidney, heart, and lung, has demonstrated its value for improving the performance of organs after transplantation [12–14]. It can be performed at hypothermic but also normothermic temperatures which allow for metabolic activity and functional examination of organs [15–18]. Administration of cells to isolated organs during machine perfusion prevents the overshoot of cells to unwanted sites in the patient. In addition, when performed under normothermic conditions, it enables the metabolic activity of administered cells, supporting their migratory, adhesive, and functional properties.

We present a narrative review of the current state of regenerative cell therapy for the intestine, kidney, liver, lung, and heart using the PubMed database as a resource for the article search. These organs are prime targets for regeneration. Their distinct structure and cellular organization allow for the comparison of different cell sources, delivery routes, and engraftment efficiencies characterizing each cell therapy approach. Furthermore, all these organs are used for transplantation, which allows the application of cell therapy in a controlled environment during ex vivo preservation. Finally, we discuss strategies to enhance the engraftment of administered therapeutic cells as well as the current challenges and future directions of the field.

Main text

Methodology

We searched PubMed for literature for this narrative review. Details of the search are summarized in the methods box below.

Search terms	Cell administration, cell therapy, engraftment, heart, intestine, kidney, liver, lung, machine perfusion, mesenchymal stromal cell, stem cell, transplant organ
Time window	Any period
Inclusion	Studies investigating administration of cells with the aim to repair the intestine, kidney, liver, lung, or heart Studies with parenchymal cell types, if not available, studies with MSC Studies including data on cell engraftment Studies including data on (absence of) effects on organ function
Exclusion	Studies solely focused on extracellular vesicles or secretome Studies solely focused on immune modulation

Cell therapy strategies for intestine regeneration

The revolutionary identification of stem cells within tissues forms the basis of cellular regeneration studies. The group of Clevers, after identifying the stem cells for the intestinal epithelium marked by Leucine-rich repeat-containing G protein-coupled receptor 5 (LGR5) expression [3], developed a methodology to isolate, expand, and culture them into intestinal organoids [8, 19]. The intestinal epithelium is a suitable target for cell replacement therapy due to its accessibility via the lumen. Early work showed that colon epithelial cells derived from dissociated intestinal organoids can engraft long term in injured mouse colon and ameliorate colitis after administration by enema [20]. Intestinal epithelial organoids can also be transplanted as a whole into the luminal space of the colon of experimental animals [21]. A mouse model of irradiation-induced bowel injury demonstrated that whole colonic epithelial organoids administered by enema engrafted for up to 4 weeks [22]. The implanted cells proliferated and differentiated into different colon cell types, improving colonic epithelial integrity. In

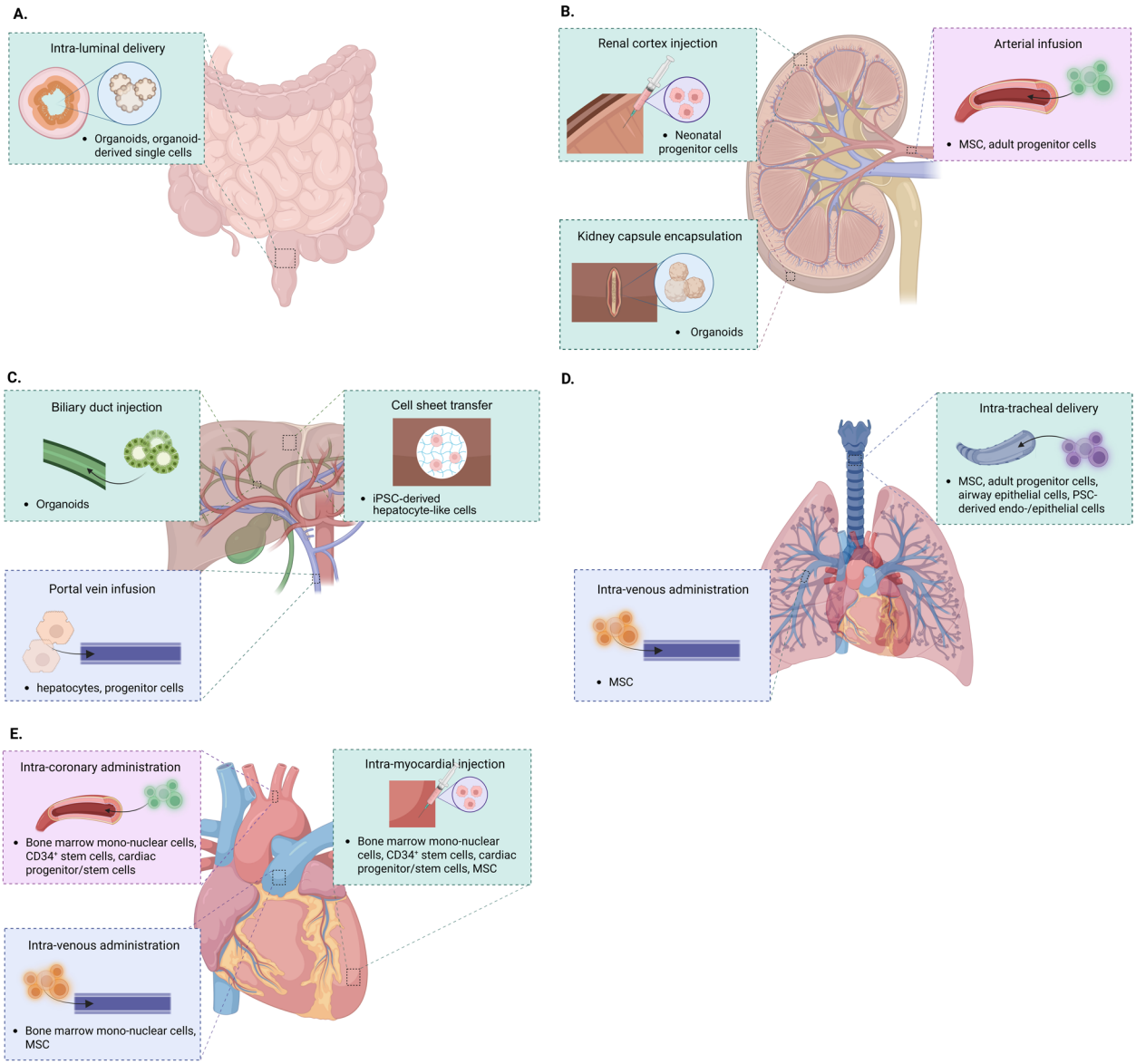

Fig. 1 Routes of cell administration for different organs. Depending on their anatomy, organs have different routes of access. While infusion-based routes ensure broad delivery of cells, intra-tissue injections can deliver cells across vascular barriers. The choice for a particular route of cell administration will also depend on the localization of the cell type to be replaced. Image created using BioRender software

addition, proof-of-concept work in a rat model demonstrated repurposing of the colon by cell removal and subsequent replacement by organoids of the small intestine [23]. Noteworthy to mention is that intestinal cell administration is typically performed in immunodeficient or otherwise syngeneic recipients. In the clinical situation, this translates to the use of autologous cells. According to reports, clinical trials using intestinal epithelial organoids have commenced, but no published results are available yet. The behavior of human intestinal epithelial organoids after implantation has been studied in a xenotransplantation model, in which human pluripotent stem cell-derived intestinal organoids containing both epithelial and mesenchymal cells were administered into the colon of immunocompromised rats [24]. These organoids were shown to be capable of reconstituting multiple cell types, not only in the mucosal layer but also in smooth muscle and vascular endothelium layers up to 10 weeks after administration, demonstrating their potent regenerative potential. One factor that may challenge cell replacement therapy of the intestine is its high self-renewal/replacement rate which is 3 to 5 days for gut

epithelia [25]. Only those organoid-derived cells which successfully integrate in the stem cell niche will have the potential for long-term survival and engraftment.

Cell therapy strategies for kidney regeneration

The kidney contains at least 20 specialized cell types organized in a complex structural arrangement. Kidney diseases lead to loss of cells, including tubular and endothelial cells after acute kidney injury, and loss of podocytes during diabetic nephropathy, hypertension, or glomerulonephritis [26, 27]. The loss of endothelial and tubular cells is partially reversible, whereas the loss of podocytes is permanent. The loss of functional cell types is commonly compensated for by increases in stromal cells that actively deposit extracellular matrix.

MSC have been a pioneering cell type for kidney regeneration studies. As one of the most studied cell types for cell therapy development [28], the reasons behind their popularity partly lie in their widespread distribution, including relatively assessable tissues such as the bone marrow and adipose tissue [29], and their low demands for in vitro expansion. Multiple studies attempted to treat injured native or transplant kidneys through administration of MSC via vascular routes. Among the first studies, Lange et al. demonstrated that infusion of MSC in the thoracic aorta ameliorated ischemia–reperfusion induced kidney injury [30]. MSC were found in glomerular capillaries, but as their effects were rapid, it was hypothesized that trans-differentiation of administered MSC into renal cells was not the primary mechanism of action. Other studies demonstrated that the reparative effect of MSC following ischemia–reperfusion injury of the kidney was mediated via macrophages [31]. MSC-derived exosomes have been indicated to play a role as well [32–34]. Intravenous infusion of MSC was furthermore demonstrated to be effective in repairing cisplatin-induced kidney injury [35–39], unilateral ureteral obstruction-induced injury [40, 41], rhabdomyolysis-induced acute kidney injury [42], and prevention of kidney allograft dysfunction [43, 44]. These studies failed to demonstrate robust numbers of MSC in injured areas of the kidney. Several studies demonstrated that MSC exhibit a poor biodistribution and an inability to pass through the lung micro-capillary network [45–48]. Accumulating evidence demonstrates that infused MSC present low survival rates with estimated retention rates between 0 and 5% at 72 h after infusion [45, 46]. It also appears that intravenously infused MSC are rapidly phagocytosed by monocytes and neutrophils [49, 50]. Notably, it has been indicated that the apoptosis of MSC in combination with the subsequent response of host macrophages is key for their therapeutic effects [50, 51]. It is, thus, not unlikely that reports of labelled MSC in injured kidney tissue are in fact immune cells that have previously phagocytosed the labelled MSC and subsequently migrated to sites of injury. A way to avoid the lung barrier is to administer MSC via the renal artery. After arterial administration, MSC appear to survive longer. Eight hours after administration of 10×10^6 MSC in a pig kidney, $1–4\times10^4$ cells per gram kidney tissue were detected with a viability of 70% [52]. However, after 14 days, most of the cells were cleared. As a result of these studies, the therapeutic effect of MSC, as reported across diverse preclinical models, is mainly attributed to their indirect effects on immune cells and to their secretome.

While targeting the kidney through the vascular system in situ is difficult, ex situ kidneys can be easily accessed via the renal artery. It has been shown that MSC delivered intra-arterially accumulate primarily in the glomeruli [52, 53]. Thompson et al. explored the effects of intra-arterial delivery of 50×10^6 multipotent adult progenitor cells to normothermically perfused kidneys and demonstrated that cell administration reduced the expression of injury and inflammatory factors and improved blood flow [54]. After 7 h of perfusion, the fluorescent label of the administered cells was detected in the glomeruli and in the interstitial space. However, the signal did not co-localize with nuclear staining, raising doubts about whether the label was retained in the administered cells. In a similar experimental setup, Pool et al. determined reduced levels of the injury molecules neutrophil gelatinase-associated lipocalin and lactate dehydrogenase after administration of 10×10^6 MSC to pig kidneys [55]. Lohmann et al. demonstrated the safety and feasibility of administering 10^7 MSC to pig kidneys during normothermic machine perfusion in an autotransplantation model but did not detect any beneficial effect [56]. A study in which MSC were administered to rodent, pig, and human kidneys during hypothermic perfusion revealed similar results, demonstrating detainment of MSC in the glomeruli and no effects on the kidneys during 2 h of warm reperfusion [57]. Collectively, these studies suggest that the delivery of MSC to kidneys during machine perfusion is safe. Nevertheless, there appears to be a limitation to the number of cells that can be safely infused as administration of 10^8 cells led to an immediate reduction in renal perfusion and showed signs of instant blood-mediated inflammatory reaction and glomerular and tubular damage 2 weeks after administration [58]. Furthermore, although some reduction of kidney injury markers was reported, convincing therapeutic effects of MSC administration are currently lacking, and there is no strong evidence of engraftment.

For cells to engraft and contribute to kidney function, more specialized cell types may be required. Culture-expanded neonatal kidney progenitor cells injected into

the neonatal renal cortex have been demonstrated to be capable of generating functional tubular structures [59]. Such treatment failed in the adult renal cortex due to massive bleeding. Several groups have explored the implantation of whole kidney organoids, mostly under the kidney capsule, to introduce multiple kidney cell types such as tubular, glomerular, and stromal cells [60]. There is, however, no evidence that subcapsular implanted organoids integrate with the underlying nephron structures or collecting duct, and, as a result, organoids do not contribute to the kidney's filtration function. Administration during machine perfusion is an attractive route for specialized cell types as they can be delivered close to their anatomical location. Kidneys appear less resilient to the conditions of machine perfusion than other organs, and normothermic perfusion has so far been limited to several hours [15, 16, 61, 62], which limits the possibility for long-term monitoring and functional assessment. Interestingly, it was recently demonstrated that kidneys can be kept under sub-normothermic conditions for 4 days [63], which provides sufficient time for cell administration and engraftment studies. A recent study demonstrated that human-induced pluripotent stem cell (iPSC)-derived kidney organoids infused in porcine kidneys during normothermic machine perfusion are still detectable in the cortex at 48 h after autotransplantation of the kidneys [64].

Although intra-arterial administration brings specialized kidney cells close to their anatomical location, tubular cells and podocytes would still need to cross an endothelial barrier. For tubular cells, an alternative route of administration could be based on retrograde injection via the ureter. This route would bypass the endothelial barrier and offer the possibility of cell delivery directly at the required location. This route is accessible during ex situ perfusion of kidneys. Urethral administration of tubular cells has not been reported yet, but it is a route worth exploring. The urethral route could potentially also allow the delivery of podocytes to their correct location at the end of the tubular system.

Cell therapy strategies for liver regeneration

Liver cell replacement strategies have been studied as an alternative to liver transplantation for five decades. One of the first attempts to develop a liver cell replacement therapy dates back to 1977, when Najarian et al. transplanted hepatocytes via the portal vein and intraperitoneally to allow recovery from drug-induced liver failure in rats [65]. In the same era, it was shown that hepatocytes transplanted into the spleen preserved their phenotypic characteristics, suggesting that the spleen could be utilized as an ectopic liver [66]. In 1992, the first hepatocyte transplantations in patients were performed [67]. Following these initial steps, various efforts have been undertaken in animal models and clinical studies, studying different cell sources and delivery strategies.

Hepatocytes, as the primary parenchymal cell type of the liver, have been the focus of most cell replacement studies. The feasibility of hepatocyte transplantation has been shown in various genetic mouse models. For example, the transplantation of human hepatocytes in immunodeficient $Fah^{-/-}$ or alpha-1-antitrypsin-deficient mice resulted in the successful repopulation of the liver by the transplanted hepatocytes [68, 69]. In the clinical setting, a case report demonstrated that the infusion of 7.5×10^9 hepatocytes in the portal vein led to successful hepatocyte engraftment and survival for 11 months in a pediatric patient with Crigler-Najjar syndrome type 1 [70]. The efficacy of clinical hepatocyte transplantation through portal vein infusion has also been demonstrated in other liver conditions, including phenylketonuria and ornithine transcarbamylase deficiency [71, 72]. Delivery of hepatocytes through infusion via the splenic artery has been described to temporarily rescue (sub)acute liver failure and facilitate bridging to liver transplantation [73]. In this study, five cell-treated patients were successfully bridged to liver transplantation, while four patients with equal disease severity who did not receive cells died within 3 days.

Although significant progress has been made in the field of hepatocyte transplantation, limitations and challenges persist [74]. Although hepatocyte transplantation can result in the amelioration of liver diseases, its effect has been shown to be transient, primarily serving as a bridge to liver transplantation rather than a permanent treatment for the disease. Most clinical studies have been based on case reports or small cohorts with limited long-term follow-ups. Hepatocyte deliveries through portal vein or peritoneal infusion have led to (lethal) vein thrombosis in some cases [75]. In addition, difficulties in the isolation and expansion of functional hepatocytes, together with the scarcity of livers as a source of hepatocytes, pose further challenges for advancing hepatocyte transplantation.

The use of progenitor cells may be an alternative for liver cell replacement therapy, given their high proliferative capacity. Transplantation of fetal human hepatoblasts in athymic mice has been shown to result in up to 10% repopulation, with engrafted cells exhibiting marker expression characteristic of adult hepatocytes, suggesting the efficient differentiation of the transplanted hepatoblasts into a mature phenotype [76]. In another study, the transplantation of human fetal liver progenitor cells led to a reduction in liver fibrosis. Interestingly, after injury resolution, the engrafted cells appeared to be quiescent and showed no evidence of neoplasia during a 9-month

follow-up [77]. Despite some promising animal-based studies, the use of fetal liver progenitor cells is hindered by ethical concerns on fetal tissue acquisition and their debatable degree of differentiation into mature, highly functional and proliferative hepatocytes. The clinical application of fetal liver progenitor cells has been limited to small cohorts, focusing mainly on proof-of-concept and safe-to-use approaches with short follow-ups of up to 1 year [78].

The use of human iPSC has been investigated as an alternative that overcomes the ethical challenges of fetal liver progenitor cells while also functioning as an "infinite" cell source and offering possibilities for autologous cell replacement approaches. In 2015, using an acute liver failure rat model, Ramanathan et al. showed that the transplantation of human iPSC-derived hepatocyte-like cells resulted in increased survival rates [79]. In another study, Nagamoto et al. engineered iPSC-derived hepatocyte-like cell sheets and placed them on the surfaces of injured livers in mice. Researchers reported reduced engraftment in organs other than the liver, increased albumin production compared to intrasplenic transplantation, and amelioration of CCl_4-induced acute liver injury [80]. Overall, iPSC-based therapeutic approaches constitute a rapidly evolving field; however, the pathway to clinical translation remains fraught with hurdles, including the risk of tumorigenesis, technical difficulties in scalability, debates over differentiation efficiency, and a lack of reproducibility.

Organoid technology is making its first pioneering steps in liver cell replacement therapy. In a study by Sampaziotis et al., extrahepatic cholangiocyte organoids were transplanted into immunodeficient mice [81]. Following transplantation under the kidney capsule, extrahepatic cholangiocyte organoids self-organized into bile duct-like structures while also expressing key biliary markers. In addition, when seeded on biodegradable scaffolds, the organoids formed tissue-like structures retaining biliary characteristics, and transplantation in an extrahepatic biliary injury mouse model led to biliary epithelium repair and gallbladder wall reconstruction. Another breakthrough study by Sampaziotis et al. in 2021 described for the first time the transplantation of organoids into a human liver during normothermic machine perfusion. After the injection of gallbladder organoids into the intrahepatic biliary duct network, the transplanted organoids successfully integrated into the intrahepatic biliary tissue, revealed upregulated expression of intrahepatic markers, and resulted in increased bile production with increased pH, indicating functional engraftment [82]. This work demonstrates the potential of cell replacement therapy for biliary repair.

Cell therapy strategies for lung regeneration

The lungs contain hundreds of millions of alveoli that are composed of a diverse airway epithelium surrounded by an extensive capillary network. This structure comprises over 40 distinct cell types, including basal cells, club cells, alveolar type 1 and type 2 epithelial cells, and pulmonary endothelial cells, fibroblasts, and a variety of immune cells. Each of these cellular compartments plays a crucial role in maintaining pulmonary function, immune defense, and tissue homeostasis. In lung transplantation, 50–80% of donor lungs are considered unsuitable for use, primarily due to various forms of acute lung injury. Acute lung injury, whether caused by infection, aspiration, ischemia, or mechanical ventilation, results in the loss of function or apoptosis of both epithelial and endothelial cells, thereby disrupting alveolar epithelial fluid transport and lung fluid balance [83]. Current strategies for repairing injured donor lungs during ex vivo lung perfusion primarily aim to modulate inflammation and strengthen endothelial barrier integrity, rather than achieving cellular engraftment [84, 85].

Several ex vivo lung perfusion studies using MSC have focused on lungs deemed suitable for transplantation or exposed to injury such as endotoxin [86], prolonged cold ischemia [87], or aspiration [88, 89]. A foundational study by Lee et al. [86] demonstrated that human bone marrow-derived MSC, delivered via the trachea to ex vivo perfused human lungs injured with *Escherichia coli* endotoxin, improved alveolar fluid clearance and reduced endothelial permeability 4 h after administration. Interestingly, similar benefits were observed with MSC-conditioned medium, suggesting that secreted factors such as keratinocyte growth factor were key mediators of the therapeutic effects rather than the direct engraftment of the cells. Notably, no engraftment of MSC was detected in the lung tissue, highlighting the paracrine rather than replacement role of these cells, albeit engraftment may require a longer period of time. Follow-up work by McAuley et al. confirmed these results in lungs deemed unsuitable for transplantation [90]. After administration of MSC during ex vivo lung perfusion, increased alveolar fluid clearance was observed within 4 h. Further murine studies by Fang et al. extended these findings, demonstrating improved survival and reduced histological injury when MSC were delivered intratracheally several hours after injury [91].

In a recent preclinical study, Edström et al. demonstrated that intravenous administration of amniotic fluid-derived MSC during ex vivo lung perfusion significantly attenuated inflammation and improved posttransplant lung function in a porcine model of endotoxin-induced injury [92]. Treated lungs exhibited reduced levels of pro-inflammatory cytokines, improved oxygenation, and

better histological preservation compared to the control group. Although amniotic fluid-derived MSC were detected in the lung tissue during ex vivo lung perfusion and within the first 24 h post-transplantation, no cells could be found by day 3 post-transplantation. Despite the lack of engraftment, a significant immunomodulatory effect, particularly on neutrophils and T cells, was observed within the first 24 h. This could mean that amniotic fluid-derived MSC remain active in the tissue during this critical early phase, but the effect could also be derived from factors released by apoptotic MSC. Overall, these findings support the paradigm of mesenchymal stromal cell-mediated paracrine immunomodulation as a therapeutic mechanism for lung repair.

Other cell types, such as multipotent adult progenitor cells, have also been tested in human and porcine ex vivo lung perfusion. Similar to MSC, these cells reduced inflammatory cell infiltration and cytokine levels in bronchoalveolar lavage fluid [93, 94], although physiologic improvements were less consistent. For instance, while reduced inflammation was evident, changes in oxygenation or compliance were not always significant.

While the paracrine effects of MSC and multipotent adult progenitors hold therapeutic promise, the restoration of lost or damaged lung tissue through the engraftment and integration of stem or progenitor cells remains a future goal. In this context, ex vivo lung perfusion provides a unique and controllable window for administering cell therapy. Unlike the in vivo setting, ex vivo lung perfusion enables targeted delivery, high-resolution monitoring, and immediate assessment of lung physiology, making it an ideal platform for testing regenerative approaches. The route of cell administration likely influences their ability to engraft. While intravenous administration may provide a more favorable environment for cells to exert their paracrine effects, intratracheal delivery enables administered cells to reach their intended alveolar engraftment site. There is evidence for flattening of MSC onto the epithelium after intratracheal delivery, although there is no proof for engraftment or differentiation [95]. In a study by Guenthart et al. [96], targeted epithelial bioengineering was explored by delivering labelled MSC or airway epithelial progenitors into decellularized, non-transplant-eligible human lungs during modified ex vivo lung perfusion. The cells were retained, exhibited a flattened morphology, and were distributed uniformly, providing proof of concept for future regenerative or chimeric graft development.

There is evidence of engraftment of other cell types after administration to the lung. It has been demonstrated that lung epithelial progenitor cells differentiated from pluripotent stem cells can durably engraft into the alveoli of syngeneic mice [97]. In a lung injury model, these cells not only integrated into the alveolar niche but also differentiated into alveolar type 1 and type 2 cells and maintained function for up to 1 year. In the study by Kolesnichenko et al. [98], the authors investigated the therapeutic potential of embryonic stem cell-derived endothelial progenitor cells in a murine model of bronchopulmonary dysplasia. They found that intratracheal administration of these cells prevented alveolar simplification and preserved lung architecture. The cell treatment enhanced vascular density and reduced inflammation, indicating that embryonic stem cell-derived endothelial progenitor cells facilitate alveolar and vascular development through paracrine and reparative mechanisms. These studies provide evidence that pluripotent stem cell-derived lung epithelial cells can achieve long-term, stable engraftment in a host.

Cell therapy strategies for heart regeneration

The heart has been a prime focus for cell therapy studies over the past 15 years. Multiple preclinical and clinical cell therapy studies for cardiac repair after myocardial infarction have been carried out, many of which have unfortunately failed to demonstrate clinically relevant beneficial effects on cardiac function [99–101].

Multiple animal studies as well as clinical trials have pointed out that the retention of cells administered to the heart is very low, regardless of the delivery route, varying from 1 to 20% even within hours after administration, as thoroughly reviewed by Li et al. [102]. These numbers are even lower long term. The low retention rates have been attributed to reasons such as cellular washout, cell death, and immune responses targeting the administered cells. In contrast to the epithelial and endothelial cells that are targets for replacement therapy for kidney, liver, and lung repair, the target cell for cardiac repair lies beyond the vessel wall. This brings about additional challenges. In contrast to immune cells and cancer cells, tissue cells cannot normally transmigrate from the vessel lumen into the tissue. To achieve efficient tissue delivery, cell administration through direct tissue injections can be utilized. There is ample expertise in intramyocardial injection of cells. Preclinical and clinical studies have repeatedly demonstrated that such injections result in low cell retention, even within the first few hours after injection. Besides direct intramyocardial injection, intra-venous and intracoronary administration methods have been explored as well but failing to ensure sufficient cell engraftment. The main cell types that have been used for cardiac cell therapy include MSC, cardiac progenitor cells, bone-marrow mononuclear cells, and CD34 + stem cells [102].

One of the main factors attributing to poor cell engraftment may be the contractile properties of the heart, which can potentially damage the administered cells or

push them out via the injection site. Another issue may be the limited capacity of injected cells to adhere to the available matrix, thereby missing important survival cues. There is evidence that intracardiac cell administrations can improve cardiac function; however, this effect appears to be independent of cell engraftment, as nonviable cell debris and non-cellular inflammatory molecules can also achieve this effect [103]. The mechanism of action likely depends on the immunomodulatory effects induced by the treatments, which inhibit injury progression rather than actively promoting repair. Repairing the heart by intracardiac cell injections longer after injury is ineffective [104]. Other disadvantages of intramyocardial injections include the method's invasiveness and restricted availability of tissue areas for treatment. The low retention rate of cells administered through intravenous and intracoronary injections results from the flush out of cells, which subsequently end up at unwanted locations.

A potential alternative approach to delivering therapeutic cells into cardiac tissue involves leveraging the increased vessel wall permeability and loosening of inter-endothelial junctions that occur following injury, thereby facilitating the migration of administered cells into the affected area. Pretreatment to increase vessel wall permeability may be applied to ex situ hearts to facilitate transendothelial migration of therapeutic cells. However, caution is required as such pretreatment may increase the heart's accessibility for immune cells too. To date, to our knowledge, there have been no studies exploring the delivery of therapeutic cells to ex situ perfused hearts.

Strategies to enhance the engraftment of regenerative cells

A major challenge in cell replacement therapy is achieving sufficient engraftment of administered cells at the correct site. Following injury, basement membranes may become vacant and offer free access to administered cells to their intended adhesion site. However, as discussed above, for certain cell types, the target engraftment site lies across the endothelium. In these cases, regenerative cells must migrate across the host endothelium at the right location to reach their destination. In contrast to cells of the immune system which efficiently transmigrate into injured tissues, healthy parenchymal cells do not have the capacity to transmigrate across the endothelium barrier. Studies have demonstrated that intravascular administered MSC fail to cross the endothelium and eventually die within the vessel lumen [46, 50, 105]. The lack of transendothelial migration of administered cells is one of the major hurdles of intravenously applied regenerative cell therapies. One potential solution could lie in transiently inducing a transmigratory apparatus in therapeutic cells. This should include the introduction of adhesion molecules to mediate selective adhesion to activated endothelium, and proteins with a trans endothelium migratory role, allowing administered cells to reach their target site and subsequently engraft and exert their therapeutic function.

Scaffolds

When regenerative cells cannot be delivered at their site of engraftment or do not have the ability to migrate across the endothelium to reach their engraftment site, biodegradable scaffolds may be used to improve the engraftment of cells. The use of scaffolds has been widely studied in hard tissue regeneration [106], but their implementation in soft tissue engineering has been limited so far. Hydrogels have been studied as carriers for growth factors or drugs and for encapsulation of cells [107]. Various hydrogels have been tested in vitro for their ability to support the growth of diverse cell types and organoids [108]. Transplantation of hydrogel-supported hepatic organoids in the abdominal cavity has been demonstrated to prolong the survival of mice with liver failure [109]. Challenges however remain to develop hydrogels that have the properties to endure physiological stress in their in vivo location; allow cell migration, proliferation, and differentiation; and avoid immune rejection [110].

An alternative approach to the use of artificial hydrogels is the use of tissue-derived extracellular matrices as a scaffold for cell adhesion. Decellularization protocols that remove cells while leaving extracellular matrix structures intact have been developed for multiple organs [111–113]. Ex situ machine perfusion provides a safe and controllable environment for applying decellularization protocols, thereby priming organs for cell therapy. It will, however, be highly challenging to replace the multitude of cell types present at distinct locations in organs after whole-organ decellularization. It may therefore be more feasible to replace cells of partially decellularized organs, such as the kidney, by selective removal and replacement of kidney endothelial cells. It has been demonstrated that rat kidneys can be successfully de-endothelialized ex situ, re-endothelialized with human immune cells and transplanted [114]. As animals were euthanized after 90 min, longer-term effects remain to be investigated.

Bio-printing

State-of-the-art bio-printing technology could aid the engraftment of therapeutic cells by providing a matrix for cell adhesion. There is a wide choice of materials to mimic the environmental cues in specific tissues. A reconstruction of the kidney tubular-interstitial niche using bio-printing has recently been shown to successfully recapitulate kidney fibrosis [115]. Further

development of such models could potentially lead to the creation of implantable bio-printed tissue. An emerging technology is four-dimensional (4D) printing, in which 3D-printed scaffolds are modified by shape-shifting stimuli over time [116]. This technique enables the creation of complex structures that would otherwise require a lengthy fabrication time using conventional 3D printing techniques. Moreover, 4D printing enables the integration of functionalities that would otherwise be impossible to combine in a single construct. For example, arbitrarily complex surface nanopatterns, known to guide stem cell differentiation and provide other physical cues, can be combined with complex 3D architectures to benefit from both surface-related functionalities and the functionalities achieved through a rationally designed microarchitecture [117]. Proof of principle 4D constructs that change shape from spheres to flat patches for the repair of cardiomyocyte tissue have been developed. Such constructs are biocompatible with iPSC-derived cardiomyocytes and can potentially be used as a tool for enhancing cell engraftment [118]. Additionally, 4D printing could be used to create constructs with relatively small thicknesses but large surface areas, allowing for sufficient oxygenation and nutrition of the cells residing within a regenerative construct. This will prevent necrotic cores, which are often seen in thick constructs, from forming. Once the tissue regeneration process has progressed sufficiently for blood vessels to form, an external stimulus can be applied to initiate the process of self-folding, thereby obtaining a complex 3D structure from the initially flat regenerative construct.

Further challenges and directions

The ability to generate specific cell types in vitro and administer these to isolated organs during ex situ machine perfusion offers opportunities for the delivery of regenerative cells to organs. Many challenges remain to be addressed before cell therapies can have a genuinely significant impact on the clinical arena. Optimal delivery routes need to be determined for each organ to ensure the successful engraftment of therapeutic cells. Insufficient data availability on cell retention is currently imposing a burden on the progress of the cell therapy field.

In addition to delivery and engraftment challenges, further challenges include the long-term survival and functionality of the administered cells. Several of the discussed studies above used cells of allogeneic origin. The advantage of allogeneic cells is that cell batches of healthy donors can be prepared, cryopreserved, and made available upon demand. However, allogeneic cells are likely to induce immune responses, which are not only complicating their use for long-term engraftment purposes but may also hamper the efficacy of cell therapy due to the paracrine effects involved. The development of immune-evasive therapeutic cells may offer a solution to this issue [119, 120].

Typically, therapeutic cells are culture-expanded and exposed to significant changes in their environment upon introduction to their target organ. In addition, tissue cells rapidly undergo anoikis, a form of apoptosis, when they lose their connection to the basement membrane or the appropriate extracellular matrix [121]. While anoikis is an important mechanism for preventing tissue cells from colonizing at incorrect sites, it limits the viability of tissue cells in suspension and narrows the time window for cell administration and engraftment. Once engraftment of therapeutic cells has been achieved, the question is how long fully differentiated cells will be able to function before undergoing senescence or cell death.

A way forward may be niche restoration for therapeutic cells at their site of engraftment. In this scenario, not only are fully differentiated cells replaced but also their progenitors, thereby creating a viable population of cells that can auto-repair in the event of new injuries. Such restoration of a progenitor cell pool has been demonstrated in colonic tissue affected by Hirschsprung disease, a rare condition characterized by the inability of enteric nervous cells to colonize the gastrointestinal tract during development [122]. Ex situ administration of iPSC-derived enteric nervous system progenitors led to the integration and differentiation of these cells into neurons in colonic tissue. Another challenge is to ensure the functionality of engrafted cells. Ideally, engrafted cells should recapitulate the physiological activity of their healthy endogenous counterparts. While this field is still in its infancy, studies in Parkinson's disease animal models and clinical trials in type 1 diabetes demonstrate that engrafted iPSC-derived dopaminergic neurons [123] and β cells [124, 125], respectively, show functional improvement after engraftment.

Finally, to ensure the successful clinical translation of the developing cellular therapies, it is crucial to consider both ethical and regulatory aspects across the research and product development pipelines. Ethical issues in the field of regenerative cell therapy are diverse and range from cell source scarcity to safety and commercialization aspects of novel cell-based therapies [126, 127]. Advice from regulatory bodies on both ethical and legal requirements is necessary already during the early stages of the cell therapy development pipeline to ensure its successful clinical implementation. Thus, collaboration between cell biologists, immunologists, physicians, ethicists, and regulators will be of utmost importance for the development of effective, safe, and societally accepted regenerative cell therapies.

Table 1 Overview of selected cell therapy studies for different organs. The current status and main outcomes of each approach vary based on the cell source and delivery route used for each target organ

Organ	Cell Source	Delivery Route	Current Status			Key Findings	Engraftment		Reference
			Pre-clinical; animals	Pre-clinical; ex-situ human organs	Clinical		Yes	No	
Intestine	organoid-derived single cells	Intra-luminal administration				colitis – mice; functional improvement	x		42
	organoids	Intra-luminal administration				colitis – mice; functional improvement	x		43
						proctitis – mice; functional improvement	x		44
						bowel injury – rats; functional improvement	x		45
						bowel injury – mice; functional improvement	x		46
Kidney	MSC	Intra-arterial administration				I/R model – pigs; temporal structural integration	x		66
						machine perfusion – pig kidneys; safety, feasibility		x	70
						machine perfusion – pig kidneys; injury amelioration, immunomodulation		x	69
						machine perfusion – human; feasibility		x	71
	adult progenitor cells	Intra-arterial administration				machine perfusion – human; functional improvement		x	68
	fetal nephron progenitor cells	Renal cortex injection				acute kidney injury – mice; functional improvement	x		73
Liver	hepatocytes	Intra-splenic administration				a1-antitrypsin deficiency – mice; replacement of host hepatocytes	x		82
						fumarylacetoacetate hydrolase deficiency – mice; structural integration	x		83
		Intra-venous administration				Crigler–Najjar syndrome type I – human; partial correction	x		84
						phenylketonuria – human; temporal disease improvement	x		86
	progenitor cells	Intra-venous administration				mice; feasibility, hepatocyte phenotype differentiation	x		90
						liver fibrosis – mice; fibrosis attenuation	x		91
	iPSC-derived hepatocyte-like cells	Intra-splenic administration				acute liver injury – mice; temporal functional improvement	x		92
		Cell sheet-based				acute liver failure – rats; injury amelioration	x		94
	organoids	Scaffold-based				biliary injury – mice; epithelium repair	x		95
		Intra-bile duct				machine perfusion – human; feasibility, improved bile properties	x		96
Lung	MSC	Intra-tracheal administration				machine perfusion – human; improved function of endotoxin-injured lung		x	100
		Intra-venous administration				machine perfusion – human; improved function of ischemia-injured lung		x	104
						lipopolysaccharide-induced injury – pigs; improved function		x	106
	adult progenitor cells	Intra-tracheal administration				machine perfusion – pigs; reduced inflammation		x	107
						machine perfusion – human; reduced inflammation		x	108
	airway epithelial cells	Intra-tracheal administration				machine perfusion – human; cell retention in previously decellularized regions	x		110
	PSC-derived epithelial progenitors	Intra-tracheal administration				bleomycin-induced injury – mice; structural integration and functional differentiation	x		111
	ESC-derived endothelial cells	Intra-tracheal administration				hyperoxia-injured – mice; increased vascular and alveolar remodeling	x		112

Table 1 (continued)

Heart	bone marrow mono-nuclear cells	Intra-arterial administration				myocardial infraction – pigs	low	116
						myocardial infraction – human; functional improvement		
		Intra-venous administration				myocardial infraction – human; no significant functional change		
		Intra-myocardial injection				myocardial infraction – pigs; structural integration		
	cardiac progenitor/stem cells	Intra-arterial administration				myocardial infraction – pigs; functional improvement		
						ischemia-reperfusion injury – pigs		
		Intra-myocardial injection				ischemia-reperfusion injury – pigs; structural integration		
						myocardial infraction – rats; increased fractional area change		
	MSC	Intra-arterial administration				myocardial infraction – pigs; functional improvement		
		Intra-venous administration				myocardial infraction – rats; no significant functional change		
		Intra-myocardial injection				myocardial infraction – pigs; functional improvement		
	CD34$^+$ stem cells	Intra-arterial administration				non-ischemic dilated cardiomyopathy – human; functional improvement		
		Intra-myocardial injection				non-ischemic dilated cardiomyopathy – human; functional improvement		

The gray shading indicates the status of the treatment (pre-clinical animals, pre-clinical ex situ organs, or clinical)

Limitations of this review

The present review provides an overview of the state of the art of cellular therapies for organ repair. The review was not set up as a systematic review, and therefore, there is risk for bias in article selection. Not all aspects of regenerative cellular therapy could be covered, and attention was focused on cell replacement vs paracrine mechanisms of action of cellular therapies, routes of administration, and cell retention, while less attention was given to cell production, as well as ethical and regulatory aspects of cell therapy.

Conclusions

In conclusion, regenerative cell therapy exists in multiple forms. The current status and outcomes of each cell therapy approach differ based on the cell source and delivery route used for each target organ (Table 1). Some regenerative cell therapies do not involve the engraftment of therapeutic cells but instead rely on the stimulation of resident cells by the administered ones (Fig. 2). These therapies have mostly short-term effects. On the contrary, the advantage of therapies based on therapeutic cell engraftment is the offered possibility for long-term effects. At the same time, the option to modify

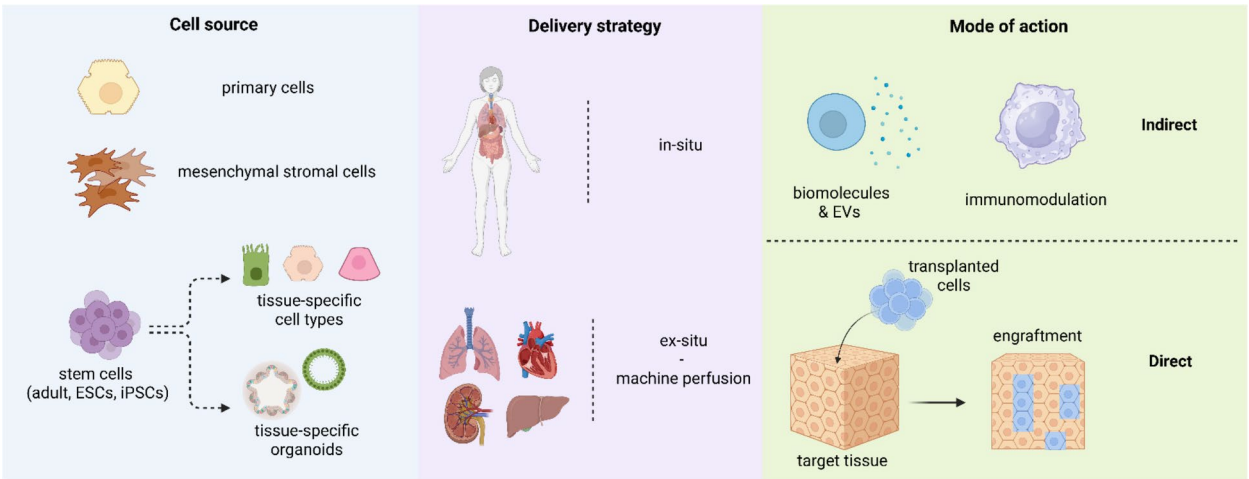

Fig. 2 Key aspects of regenerative cell therapy: cell source, delivery strategy, and mode of action. Different cell types have been used for the development of regenerative cell therapies. They can be administered either in situ to patients or ex situ to isolated organs during machine perfusion. Their effects can be indirect via stimulation of resident cells or direct via tissue engraftment and replacement of lost or injured cells. Image created using BioRender software

the properties of the administered — and subsequently engrafted — cells could aid the regeneration of target organs by using cells with potentially improved function.

Cellular regenerative therapies are more feasible for some organs than for others. This is due to differences in the anatomy of target organs and differences in the properties of therapeutic cells. The luminal structure of the intestine offers access to the epithelial lining of the intestine and makes epithelial cell replacement feasible. Although less easily accessible than the intestinal lumen, the epithelial structures of the kidney tubules and liver bile ducts are exposed to fluid to which cells can be administered in direct proximity to their intended site of action. It does not require migration of the cells to reach their site of engraftment. Cells that are localized across an endothelial barrier and cells that do not face a lumen, such as kidney podocytes and cardiomyocytes, are more difficult to replace. In addition, cell types such as podocytes and cardiomyocytes show less proliferative potential than, for instance, epithelial cells of the intestine, kidney tubule, and lung alveoli, which is likely having an impact on their regenerative effects. As a result of these differences between organs, it is expected that the development of regenerative cell therapies will progress at different rates for different organs.

Machine perfusion technology offers possibilities for the administration of cells for organ regeneration. While proof of principle has been demonstrated, further research is needed to generate fully functional cells for administration, facilitate engraftment, monitor long-term survival and functionality, and establish long-term safety. The more advanced status of cell replacement therapies for neurological disorders and diabetes could guide the further development of such therapies for organs such as the intestine, kidney, liver, lung, and heart.

Abbreviations

4D	Four-dimensional
iPSC	Induced pluripotent stem cell
LGR5	Leucine-rich repeat-containing G protein-coupled receptor 5
MSC	Mesenchymal stromal cells

Acknowledgements
Not applicable.

Authors' contributions
LP, SL and MH wrote the manuscript. All authors read and approved the final manuscript.

Funding
The authors thank Convergence Health & Technology, the alliance between Erasmus Medical Centre Rotterdam, Erasmus University Rotterdam, and Delft University of Technology, for their financial support in this research.

Data availability
No new data was generated for this manuscript.

Declarations

Ethics approval and consent to participate
The manuscript includes no human or animal studies which require ethics approval.

Consent for publication
All authors consent to the publication of this manuscript.

Competing interests
The authors declare no competing interests.

Received: 24 July 2025 Accepted: 11 February 2026
Published online: 25 February 2026

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