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# Cellular modifications and biomaterial design to improve mesenchymal stem cell transplantation

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Research has advanced considerably since the first clinical trial of human mesenchymal stem cells (MSCs) in the early 1990s. During this period, our understanding of MSC biology and our ability to expand and manipulate these cells have provided hope for the repair of damaged tissues due to illness or injury. MSCs have conventionally been injected systemically or locally into target tissue; however, inconsistent cell homing and engraftment efficiencies represent a major bottleneck that has led to mixed results in clinical studies. To overcome these issues, MSCs have been pre-conditioned with biomolecules, genetically altered, or surface engineered to enhance their homing and engraftment capabilities. In parallel, a variety of cell-encapsulating materials have been designed to improve cell delivery and post-transplantation survival and function. In this review, we discuss the current strategies that have been employed on cultured MSCs to improve targeted cell delivery and retention for tissue repair. We also discuss the advances in injectable and implantable biomaterial technologies that drive the success of MSC-based therapies in regenerative medicine. Multi-faceted approaches combining cellular modification and cell-instructive material design can pave the way for efficient and robust stem cell transplantation for superior therapeutic outcomes.

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## Introduction

Throughout life, multicellular organisms maintain healthy homeostasis by regulating a delicate balance between cell regeneration, replacement, and death. However, exposure to disease, injury, infection, and/or age can upset this balance. While some tissues, such as the epithelial lining of the intestine or skin, are more resilient to such stresses due to their superior regenerative abilities, other tissues such as cardiomyocytes are challenging to regenerate. When tissues are damaged beyond their intrinsic repair capabilities, external interventions are required to promote tissue regeneration and regain tissue function.

Over the past two decades, stem cells have gained significant attention with the potential to renew damaged tissues and enhance tissue function. Of the various stem cells reported in the literature, mesenchymal stem cells (MSCs), also known as mesenchymal stromal cells, have found greatest clinical application in cell therapies.[1] The attraction of MSCs lies not only in their biological ability to self-renew, to secrete tropic factors, and to differentiate into multiple mesodermal lineages, but also in their accessibility from various tissue sources (*e.g.* bone marrow, adipose tissue, placenta, umbilical cord, dental pulp), economics of expansion and transplantation, reduced immunogenicity,[2] and limited ethical considerations compared to their embryonic counterparts.[3] Mesenchymal stem cells have been explored in pre-clinical to clinical therapies, for neuropathies (spinal cord injury, Parkinson's disease, Alzheimer's disease), cardiomyopathies, diabetic nephropathies, cancer, and other disorders of the eye, bone, skeletomuscular tissue, cartilage, and skin.[4,5]

To standardize the implementation of stem cell therapies, a reference plan was established called DOSES: D – donor (autologous, allogenic or xenogeneic), O – origin of tissue, S – separation method, E – exhibited cell behaviour, S – site of delivery.[6–9] A search for clinical trials (**https://clinicaltrials.gov**) from 2015 to 2023 using keyword "mesenchymal stem cells" results in about 963 trials. Of these, 657 studies have been initiated in the last five years (since 2018), with more than 60% in phase 1 and 2, clearly showing the rapid emergence of MSCs as a therapeutic modality. While North America and China have been the leaders in the conduct of MSC clinical trials, South Korea, Japan and India have approved more MSC clinical products than other countries[10] (Table 1). A number of recently published review articles have discussed the timeline of MSC clinical therapies.[9–13]

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Table 1 Commercially available MSC-based therapeutic products targeted to various conditions

| Name | MSC type | Target condition | Formulation | Administration route | Country | Market approval |
|---|---|---|---|---|---|---|
| Prochymal | *Ex vivo* cultured allogenic BM-MSCs | Acute GvHD in children | 100 million cells | Intravenous | Canada | 2012 |
| Ixmyelocel-T | Autologous BM-expanded multicellular cells | Heart failure due to DCM | 35 to 295 million cells[14] | Intramyocardial | USA | 2017 |
| Alofisel | Allogenic adipose-derived MSCs | Complex anal fistulas in adults with Crohn's disease | 120 million cells | Locally in fistula region | European Union | 2018 |
| Cellgram-AMI | Autologous BM-MSCs | Acute myocardial infarction | 50 million cells/10 mL, 70 million cells/14 mL, 90 million cells/18 mL | Intracoronary artery | South Korea | 2011 |
| CARTISTEM® | Allogenic UCB-MSCs | Knee cartilage defects with osteoarthritis | 2.5 million cells/500 μL $cm^{-2}$ (size of knee cartilage defect) | Locally *via* surgical method or arthroscope | South Korea | 2012 |
| Cupistem | Autologous adipose-derived MSCs | Crohn's fistula | 30 million cells per mL | Locally in fistula region | South Korea | 2012 |
| NeuroNata®-R | Autologous BM-MSCs | ALS | 40 million cells/4 mL | Intrathecal | South Korea | 2014 |
| TEMCELL® HS | Allogenic MSCs | Acute GvHD | 72 million cells/bag (10.8 mL) | Intravenous | Japan | 2015 |
| Stempeucel® | Allogenic BM-MSCs | Critical limb ischemia | 100 or 200 million cells/ 15 mL (ref. 15) | Intramuscular | India | 2016 |

MSCs – mesenchymal stem cells; BM-MSCs – bone marrow-derived MSCs; UCB-MSCs – umbilical cord blood-derived MSCs; GvHD – graft-*versus*-host disease; DCM – dilated cardiomyopathy; ALS – amyotrophic lateral sclerosis.

The clinical success of MSC-based cell therapies requires the optimisation of various *ex vivo* and *in vivo* aspects, including MSC isolation, expansion, characterization, delivery, homing, and engraftment. Moreover, MSCs are highly heterogenous in nature, containing populations with various progenitors, cell states, and functional profiles.[16] The first versions of MSC therapies have relied on the intrinsic homing ability of the injected cells to migrate to sites of injury, which can be ineffective. Recent advancements in tissue engineering and cell biology have explored more efficient delivery modalities that can enhance MSC homing and engraftment to target tissues.

Survival of MSCs following transplantation to the site of injury is another critical factor for successful MSC therapies. The harsh local microenvironment at the site of injury often reduces transplanted cell viability due to anoikis and oxidative stress. Anoikis is a form of programmed cell death caused by the loss of extracellular matrix-dependent cell adhesion. Furthermore, damaged tissues often generate imbalanced levels of reactive oxygen species relative to intrinsic antioxidative mechanisms. Therefore, strategies to improve cell engraftment, retention and survival under stress conditions are crucially needed to fully attain the benefits of MSC therapies.

In this review, we summarise the available approaches to MSC administration and implantation as part of cell-based therapies. We discuss the conventional methods of cell delivery *via* injection, outline the key processes underpinning MSC homing, and explore current strategies of enhancing MSC delivery, engraftment, and retention in target sites. Specifically, we highlight recent advances that have been made in the areas of MSC preconditioning, genetic modification, surface engineering, and biomaterial design, which have led to pre-clinical benefits and hold promise for therapeutic success in the clinic.

## Cell delivery and retention: a challenge in cell therapies

MSC therapies date back more than half a century, with the first clinical application by Thomas and co-workers in 1957,[17] and rose to prominence in the early 1990s.[18] The hype of MSCs as a "magic bullet" to treat a range of conditions gave rise to a number of unregulated "off-the-shelf" therapies.[19] One of the main reasons for this uptick in MSC products was the relative ease with which MSCs could be cultured and expanded. For instance, 25 mL of bone marrow can be propagated into about 100 million cells in two weeks, which is sufficient for a clinical dose.[8] However, clinical outcomes are not defined solely by cell dosage, but by a compounded range of factors including MSC homing, engraftment, and survival.

While injecting stem cells can be beneficial for initiating the repair of small injuries, this approach is often inadequate for severe injuries where cell-for-cell replacement is required. For instance, the human heart is the size of a closed fist,[20] and an infarct region may destroy tissue volume equivalent to that of one or two fingers, which is challenging to repair with a single dose of 100 million cells. Recovery can be further complicated by the limited engraftment and survival of injected cells, as only <1% are known to survive by 24 to 48 hours post-injection,[21] depending on the state of the disease and age of the patient.[22] These issues of cell delivery and retention are not only limited to MSC-based therapies but are also prevalent in other cellular therapies.[8]

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## Systemic and local injection of MSCs

One of the easiest and most common modes of cell delivery is *via* the intravenous (IV) route (Fig. 1). However, while it is relatively non-invasive and technically straightforward, entrapment of stem cells in other host tissues may significantly reduce clinical efficacy.[21] In a myocardial infarct animal model, IV injection of stem cells achieved modest cardiac recovery, with only a small fraction of cells engrafted in the myocardium and significant non-cardiac entrapment of cells.[23–25] While the heart may be considered a particularly challenging injection site due to its regular contractions, injecting MSCs in other organs has also led to modest benefits. For example, Liu *et al.* (2022) performed a meta-analysis study to evaluate the efficacy and safety of MSC therapies for chronic liver disease. While MSC IV injections appeared to improve biochemical markers such as albumin levels and clinical severity scores, no benefits to survival rate were ultimately observed.[26] Celis-Ruiz *et al.* (2022) recently reported on >60-year-old patients with moderate to severe stroke, who were injected with adipose-derived MSCs. After 24 months, there was no observed improvement in clinical outcomes when compared to the placebo group.[17] In another meta-study of 13 clinical studies of acute respiratory distress syndrome (ARDS), MSC treatment did not trigger adverse effects and reduced mortality rates when compared to controls; although findings may have been limited by the sample size and lack of secondary endpoints.[27] In a multicentric randomised trial for myocardial infarct reperfusion injury, IV-injected MSCs reduced ventricular tachycardia, and improved ejection fractions compared to the placebo group.[28] While other similar studies also demonstrate reversal in heart remodelling after MSC injections, the debate on their therapeutic efficacy is far from settled,[21] due to the lack of consistency between initial patient conditions and evaluation protocols and endpoints.[29] Apart from MSC entrapment in non-target tissues, the IV route of delivery is also not suited to patients

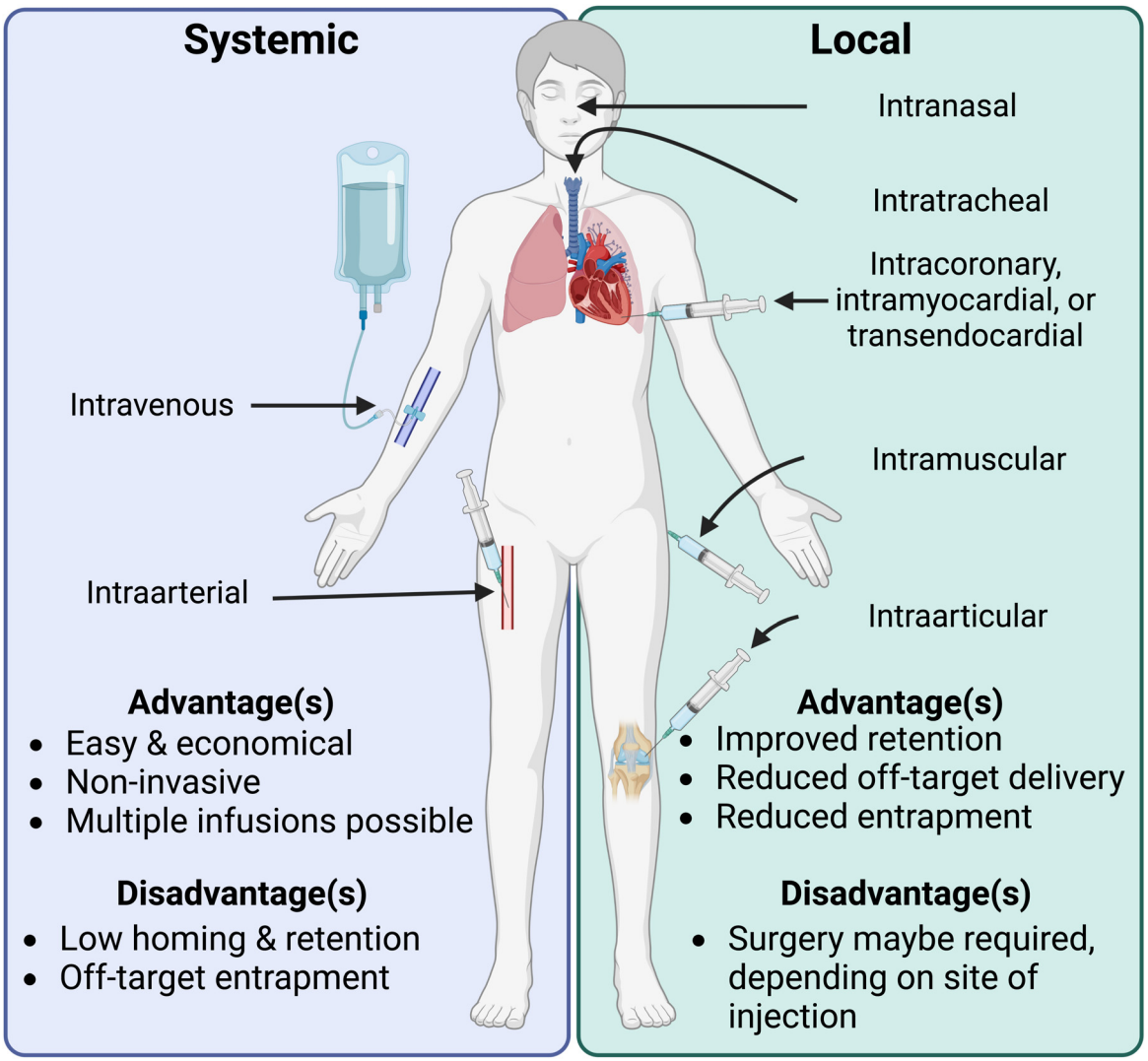


**Fig. 1** Various methods of MSC delivery for clinical applications, including potential advantages and disadvantages. Cell delivery approaches can be broadly categorised into systemic and local/targeted routes.


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with occluded arteries. Furthermore, homing and engraftment are highly dependent on internal homing signals that peak a few days after myocardial infarction, and are hence unlikely to be effective in patients with chronic myocardial infarcts.[21]

In general, while there is consensus regarding the safety of MSC administration *via* the IV route, therapeutic efficacy ranges between low to moderate in various organs due to limited retention. Jiang *et al.* (2013) demonstrated that only 0.25% of IV-delivered MSCs were retained within the myocardium in an ischemic-reperfusion model of myocardial infarction,[30] likely due to entrapment of cells in various tissues.[31,32] *In vivo* tracking of firefly luciferase-transfected murine MSCs showed that most transplanted MSCs were trapped in the lungs, due to their large size (15–19 μm) relative to capillaries (5–10 μm).[33]

Almost all studies support MSC entrapment in the lungs as an inevitable consequence of systemic infusion. This entrapment could occur not only due to the size of the MSCs but also the receptor–ligand interactions between MSCs and lung endothelia. Nystedt and co-workers showed that systemic injection of differently-sized bone marrow and umbilical cord MSCs were trapped in the lung post-injection, but clearance of umbilical cord MSCs was more rapid than that of bone marrow MSCs, suggesting that smaller cells display reduced lung retention. Furthermore, umbilical cord MSCs expressed significantly higher levels of α4 and α6 integrins, hepatocyte growth factor receptor, and general fucosylation levels than bone marrow MSCs, linking these cell surface proteins to MSC lung clearance.[34] Conversely, increased fibronectin expression by MSCs is proposed to correlate to lung adherence,[34] as is the surface expression of VCAM-1, which may mediate cell adhesion to pulmonary vasculature.[35]

In another comprehensive study in a rat lung injury model, Schmuck and colleagues (2016) determined the distribution and survival of IV-injected MSCs in different organs over a period of days. Results suggest that 82% of injected cells were found in non-target organs within an hour of injection, with the highest concentration of cells in the liver (78%), followed by the lungs (20%). Two days after injection, only 0.06% of the infused cells were detected in all organs, with the liver still retaining the highest number of cells.[36] Other studies have also shown cell accumulation in organs like the spleen.[37,38] This non-target tissue entrapment of MSCs may be promoted by coagulation reactions induced by systemic infusion of the cells.[39]

To alleviate this issue, pre-treatment of mice with sodium nitroprusside, a drug used for lowering blood pressure, has been shown to reduce lung entrapment of MSCs during IV delivery.[33] Similarly, inactivation of the VCAM-1 counter-ligand (VLA-4/CD49d) on MSCs significantly increases pulmonary passage in rats.[35] Heparin pre-treatment has also been shown to reduce lung embolisms, triggered by the administration of large doses of MSCs, and enhance migration of MSCs in a mouse model.[39] While these options significantly increase pulmonary passage, the cell numbers reaching arterial circulation are still only a small fraction of the initial administered dose.

Therefore, other systemic delivery routes, such as intraperitoneal (IP) and intra-arterial (IA) routes, have been investigated to address MSC entrapment in non-target tissues. These delivery methods bypass initial cell uptake by lungs, enhancing engraftment in target sites compared to IV injection.[37] A comparison of IV *vs.* IA administration of MSCs labelled with superparamagnetic iron oxide showed successful cell delivery and engraftment in a cerebral ischemic rat model after IA but not after IV injection, and the reduction of laser doppler flow during cell infusion correlated well with the degree of intracerebral engraftment.[40] Wang and co-workers (2013) performed a meta-analysis of 21 small animal studies using MSCs to treat impaired kidney function. Results suggested that arterial delivery of MSCs favourably reduced serum creatinine levels when compared to intravenous injections.[41] However, a study on a type I diabetic nephropathy rat model found that while 20% of IA administered MSCs localised to the injury site after 24 hours, only 3% of the cells were retained after two months.[42] Similarly, hypoxia-grown MSCs injected intra-arterially in a murine hind limb ischemia model demonstrated only 0.2% cell retention at two weeks post-transplantation,[43] indicating that the initial cell engraftment enhanced by IP or IA delivery routes does not necessarily improve longer-term retention.

To overcome issues of low cell migration and retention associated with systemic MSC administration, localised cell delivery routes (Fig. 1) such as intracoronary, intramyocardial or transendocardial injections are viable options for the heart. As cells are directly injected in the peri-infarct area, there is less reliance on their homing capability, which is expected to boost cell engraftment and therapeutic function above levels achieved *via* the IV route. Unfortunately, clinical trials on cardiac conditions with cells delivered through either intracoronary or intramyocardial routes have produced mixed outcomes.[44–47] Moreover, the efficacy of cell engraftment, as measured by a reduction in infarct size, was not observed, except in a phase I/II randomised transendocardial autologous cells in ischemic heart failure trial (TAC-HFT), where transendocardially injected cells reduced infarct size by 18.9% as compared to 5.2% in the placebo group in patients with chronic ischemic cardiomyopathy.[48] Furthermore, most clinical studies lack data on cell retention or survival that could have provided information on the fate of grafted cells. However, studies in porcine models indicate that only 3–6% of cells are retained in the heart after intra-coronary injection, and only 3–11% are retained after intra-myocardial injection.[25,49]

As with the heart, other organ-specific delivery routes have been adapted to achieve better functional outcomes. For instance, MSCs were intraarticularly injected in rats with injured anterior cruciate ligament, medial meniscus and articular cartilage. Evaluations at 4 weeks showed homing and engraftment of MSCs at the injured sites, and *in situ* differentiation aiding in tissue regeneration.[50] Similarly, in a porcine model of cartilage defect, MSCs suspended in hyaluronic acid were intraarticularly injected, and results were evaluated at 6 and 12 weeks. Marked improvements were observed at both time points based on histopathological evaluations, associated with the presence of transplanted MSCs in the host cartilage.[51] In a rodent model of bronchopulmonary dysplasia, intratra-

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cheal delivery of MSCs improved survival and attenuated alveolar and lung vascular injury. However, engraftment of cells was disproportionately low (0.1%), and the benefits were attributed to paracrine-mediated mechanisms.[52]

In general, there is substantial evidence that MSC administration *via* the systemic route is operationally straightforward, less invasive, clinically economical, and feasible for repeat infusions, but this approach carries limitations for cell homing and engraftment due to off-target effects. On the other hand, MSC administration through local routes is direct and can result in improved cell delivery due to a reduced reliance on cell homing capabilities, but post-transplantation retention and survival are highly variable and organ-dependent. Moreover, some local delivery approaches like intramyocardial or intraarticular routes would require surgery, increasing the costs associated with cell therapies. Both systemic and local delivery modalities are currently used clinically, depending on the target organ and condition, as shown in Table 1.

## Mechanisms underpinning MSC homing and engraftment

MSC delivery, *via* systemic and, to some extent, local injection methods, relies on the homing of cells to the target tissue. However, the process of homing is not yet fully understood, and there is limited understanding of the fate of injected cells. It is also debated if MSCs localize to host tissue due to passive entrapment, or if there are active factors that guide cells to specific tissues.[53] MSC homing requires modulation by chemotactic factors, extracellular matrix components,[54] and receptors like selectins and integrins.[55] The molecular mechanisms of homing are reviewed in detail elsewhere.[55,56] In brief, MSCs express cluster of differentiation 44 (CD44), which binds to selectins expressed by endothelial cells, causing MSCs to roll along the vascular wall, initiating the rolling process.[57] This is followed by an activation step that is mediated by a G-protein coupled chemokine receptor, generally in response to an inflammatory signal. One of the most well-studied homing signals is the stromal derived factor-1 (SDF-1) and C-X-C motif chemokine receptor 4 (CXCR4) axis.[58] Other factors like monocyte chemoattractant factor-1 (MCP-1) and CC motif chemokine receptor 2 (CCR2) are also known to play a role.[59] Stable activation-dependent arrest, the third step in the sequence, is regulated by integrins. SDF-1 activates integrin α4β1 (VLA-4) on MSCs, which in turn binds to VCAM-1 on endothelial cells.[60] In the next phase of transmigration, MSCs traverse the endothelial membrane by secreting matrix metalloproteases (MMPs), resulting in the breakdown of the endothelial basement membrane.[61] In the last stage of the homing process, MSCs migrate to the site of injury. There are a plethora of signalling factors and receptors that tightly control and orchestrate this complex process (Fig. 2). Detailed knowledge of these events has been used to develop various strategies to enhance homing of MSCs for clinical purposes.

## Cellular strategies for improving MSC homing and retention

Over the years, MSC homing and engraftment have been demonstrated to occur with limited efficiency,[56] potentially due to the low and heterogeneous cell expression of factors/receptors involved during this process, such as CXCR4.[62–64] *Ex*

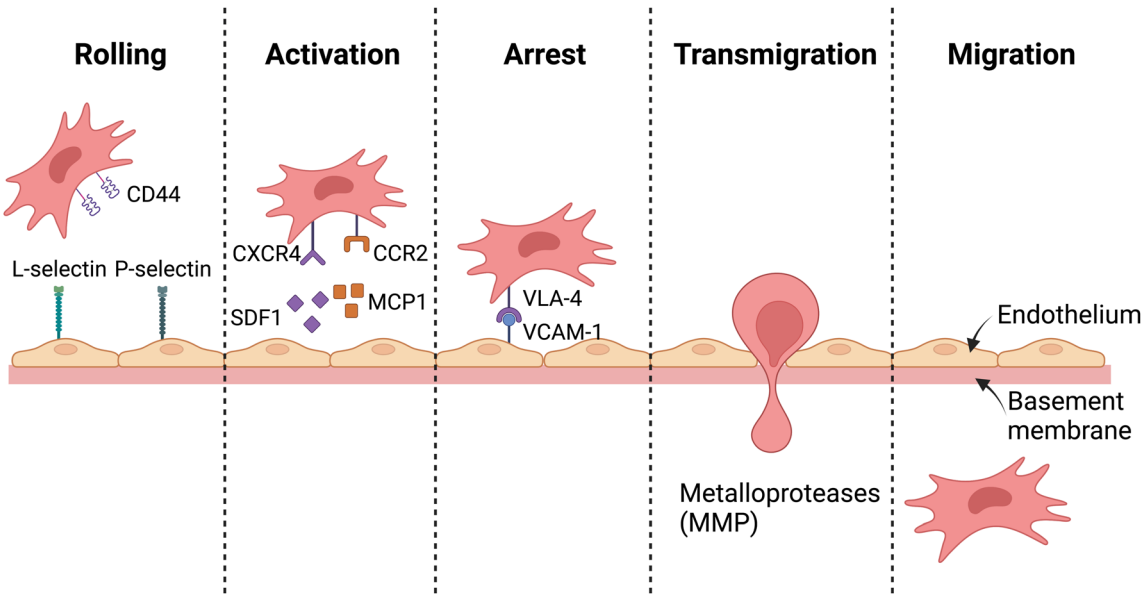


**Fig. 2** Schematic representation of MSC homing and migration, which involve five specific stages; namely, rolling, activation, arrest, transmigration and migration. The key proteins involved during each stage are indicated.


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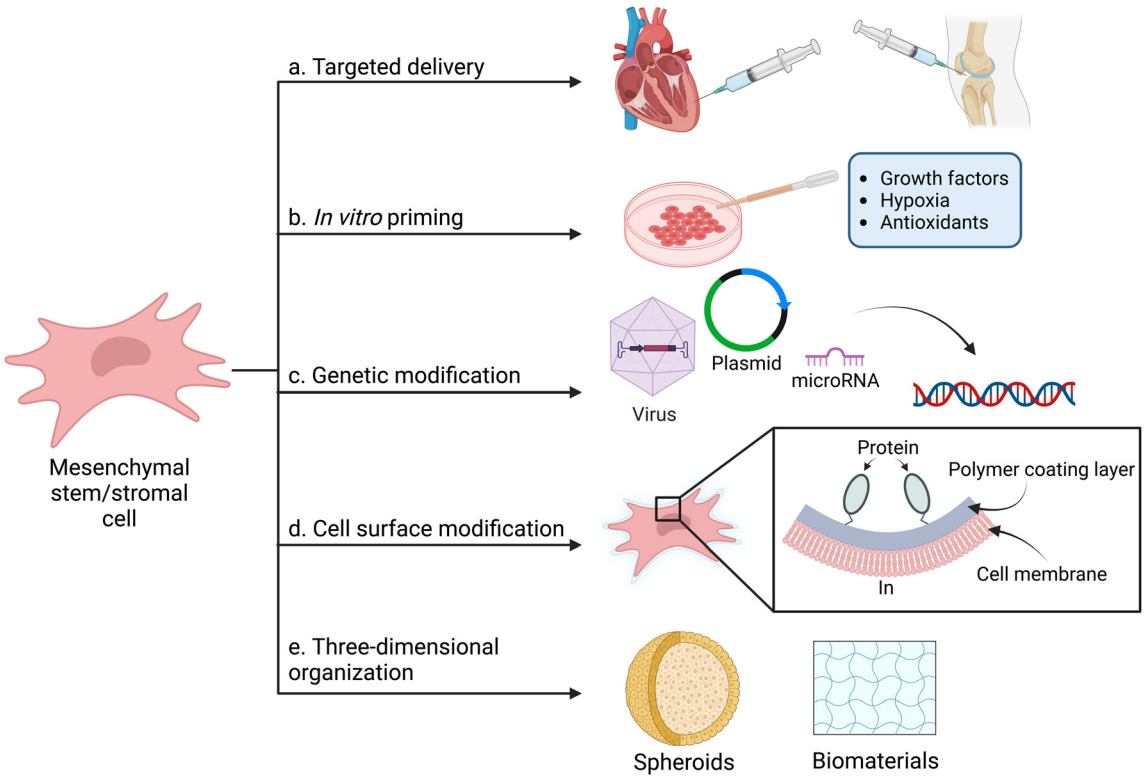


**Fig. 3** Strategies to enhance homing of MSCs, including (a) targeted delivery to specific organs, (b) *in vitro* priming of cells with different factors, (c) genetic modification, (d) cell surface modification, and (e) three-dimensional organization of cells alone or within biomaterials.

*vivo* expansion of MSCs is thought to lead to a gradual loss of these homing receptors, and MSCs of different origins may also express different receptor profiles.[65] To mitigate these issues, various strategies have been applied to enhance MSC homing and engraftment (Fig. 3). These approaches mostly target one or multiple steps of the homing process (*i.e.*, rolling, activation, arrest, transmigration, and migration).

### Pre-conditioning of MSCs

**Enhancing internal homing signals.** Since cultured MSCs downregulate the expression of homing molecules, one of the first approaches to improve homing has been to activate or enhance the levels of these molecules by the addition of cytokines, either individually or in a cocktail, during *in vitro* expansion. Expression of the major homing receptor CXCR4 can be upregulated by a cytokine cocktail, which significantly improves MSC homing to the bone marrow, compared to untreated MSCs.[66] Similarly, pre-treatment of rat MSCs with insulin-like growth factor 1 (IGF1) enhances CXCR4 expression and its migratory response to SDF-1 *via* the PI3K pathway.[67] Priming of MSCs with two mood stabilizing agents, valproic acid and lithium, also elevates levels of CXCR4 and matrix metalloprotease 9 (MMP9), respectively. Both molecules independently increase SDF-1-mediated MSC migration through different target mediators.[68] The primed MSCs show increased homing to infarcted cerebral regions, resulting in improved functional recovery, reduced infarct volume and enhanced angiogenesis.[69] Similarly, MSCs cultured with GSK3β inhibitors (LiCl, SB-415286 and AR-A014418) display increased *in vitro* migration, attributed to increased levels of migration factors, including CXCR4 and phosphor-β-PAK-interacting exchange factor (PIX).[70] In another study, interleukin 1β (IL1β) primed MSCs show upregulated CXCR4, enhanced migration to the inflammation site, and improved therapeutic efficacy in a murine colitis model.[71] Similar priming studies with deferoxamine, an iron chelator,[72] and complement component 1q (C1q)[73] have demonstrated similar results. Inflammatory cytokines like interferon-gamma (IFNγ) have also been used to prime MSCs. MSCs pre-treated with IFNγ show beneficial effects in a murine model of colitis, exhibiting better survival rates, reduced colitis scores, suppressed amyloid and proinflammatory cytokine levels, and increased body weight as compared to non-primed MSCs. Moreover, interferon treated MSCs show significantly higher (10–15%) homing to the intestine during colitis compared to untreated cells.[74]

The homing capability of MSCs can also be enhanced by culturing them in platforms that approximate the three-dimensional (3D) architecture of their native environment. Studies have shown that MSCs cultured in a 3D environment, either as spheroids or on biomaterials, tend to preserve homing, migratory and immunomodulatory capabilities that are generally lost in planar cultures.[75] It is suggested that these changes are closely regulated with the activation or inhibition of cell behaviour signalling pathways. When umbilical cord-derived

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MSCs were cultured in a 3D porcine acellular dermal matrix and injected in mice, the cells demonstrated better homing and migratory abilities to various organs due to the higher expression of toll-like receptors and CXCR4. The authors also demonstrated that 3D cultured MSCs showed reduced lung entrapment and higher host tissue engraftment than 2D cultured cells.[76] These studies indicate that MSCs can be pre-conditioned to enhance CXCR4 expression to improve homing in animal models.

**Factors improving engraftment and retention.** Priming with various growth factors to enhance MSC homing and engraftment has also been explored. Granulocyte colony stimulating factor (GCSF), a 25 kDa haematopoietic cytokine, is well-established to mobilize stem cells.[22] MSCs, pre-treated with GCSF before transplantation into a pulmonary fibrosis model, exhibit significant homing to the lungs and enhanced anti-fibrotic effects. Homing was inhibited by CXCR4 knockdown, indicating the role of this protein in GCSF-stimulated MSC mobilisation.[77] In a rat model of Parkinson's disease, co-treatment of bone marrow MSCs with GCSF improved biochemical markers of disease, and allowed the cells to cross the blood–brain barrier and engraft to the brain.[78] Furthermore, Pan *et al.* (2009) showed that administration of amniotic fluid MSCs, along with GCSF, suppresses apoptosis and inflammation, and increases survival of transplanted cells. Cells delivered with GCSF are associated with increased nerve myelination and improved motor function compared to cell preparations without GCSF.[79]

The loss of grafted cells and lack of cell integration into the host tissue may be due to a microenvironment that is unconducive to cell adhesion. Enhancing cell production of extracellular matrix components can therefore improve engraftment efficacy. MSCs primed with low levels of transforming growth factor (TGFβ1) are shown to increase expression of matrix proteins and matrix-contacting receptors, including fibronectin, collagen I and IV, tenascin-C, and integrins. When TGFβ1-primed or control MSCs were intravenously injected in a rat LPS-induced model of acute lung injury, the primed MSCs reduced the severity of injury. Furthermore, primed MSCs survived in damaged lungs until day 14 compared to untreated cells, suggesting the role of matrix proteins in increasing cell survival and retention.[80] As another example, a cocktail of fibroblast growth factor-2 (FGF2), platelet-derived growth factor-AA (PDGF-AA), forskolin and human heregulin-β1 was used to prime MSCs for 8 days and injected in a mouse model of hind limb ischemia. After transplantation, the primed MSCs significantly improved tissue perfusion and increased capillary formation, allowing the limb to be salvaged compared to non-primed MSCs. The primed MSCs also exhibited better retention and survival *in vivo*, which can be attributed to secreted pro-angiogenic and growth factors, vascular endothelial growth factor (VEGF) and hepatocyte growth factor (HGF).[81] Recently, Willer and colleagues (2022) showed that administration of human MSCs loaded with a defined set of cargo significantly accelerates wound healing in a diabetic rat model, which was characterised by increased epidermal and dermal maturation, collagen formation, vascularization, and cell infiltration.[82] The cargo consists of thirteen factors, including brain derived neurotropic factor (BDNF), epidermal growth factor (EGF), GCSF, TGFβ1, HGF, FGF2, VEGFA, leukemia inhibitory factor (LIF), prostaglandin E2 (PGE2), osteopontin, indoleamine 2,3-dioxygenase (IDO) and interleukins (IL1α and IL6). Beneficial effects were seen with repeated topical administration of cells at specific stages of wound repair, namely inflammation, proliferation, and remodelling. The human MSCs survived and were detected in the wound area interspersed with rat tissue until four days post-delivery.

In addition to growth factors, antioxidants and drugs have also been used to prime MSCs. MSCs, pre-conditioned with a natural antioxidant, fucoidan, and IV injected in a murine model of hind limb ischemia, show five-fold higher engraftment compared to non-primed MSCs. This finding suggests that pre-conditioning with antioxidants can promote cell survival and proliferation in ischemic tissues, resulting in improved functional recovery.[83] Similarly, pre-treatment of MSCs with haemin, a potent heme oxygenase-1 (HO-1) inducer, in serum-deprived and hypoxic conditions, enhances cardioprotection and improved heart function in a mouse myocardial infraction model. While both primed and untreated MSCs were detected four weeks after transplantation, the number of primed MSCs was more than two-fold higher than control cells, indicating the ability of haemin priming to promote MSC survival in ischemic tissue.[84]

**Stress-regulated homing and engraftment.** Pre-exposure of MSCs to low levels of cellular stress is another method to enhance cell homing, engraftment and post-transplantation survival in sites of tissue damage. Stress can be induced by altering the normal culture conditions of MSCs, primarily by maintaining cells in hypoxia, growing cells in serum-free conditions, or subjecting cells to oxidative stress.

Many stem cell niches are hypoxic.[85] Hence, pre-conditioning of MSCs by culturing them in hypoxia recapitulates conditions closer to their native niche. MSCs expanded in normoxic conditions can undergo stress when transplanted to hypoxic injury sites *in vivo*, which triggers premature death and reduced homing and engraftment.[86] As such, adapting MSCs to hypoxic conditions (1–5% $O_2$) may enhance their performance post-transplantation. Hypoxic conditions have been shown to increase expression of CXCR4, CX3CR1 and CXCL7, which are vital for the homing process.[87–90] Vertelov and colleagues (2013) showed that MSCs cultured in hypoxic (5% $O_2$) conditions demonstrate markedly higher targeted migration, compared to cells grown in a normoxic (21% $O_2$) environment, alongside an upregulation of growth factors (HGF, PDGF-AB, EGF, VEGF, FGF2 and IGF), chemokines (MIP1α, BCA-1, RANTES, and SDF1α) and inflammatory cytokines (IL6, IL1β and tumour necrosis factor alpha (TNFα)). The group also demonstrated that hypoxic conditions enhance the activation of RhoA,[91] a vital signalling factor in MSC migration.[85,92] Consistent with this model, hypoxia-primed MSCs displayed improved survival and retention in a murine hind limb ischemic model compared to normoxia-cultured cells, and are

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associated with enhanced vascularization and accelerated restoration of blood flow.[43] Similarly, MSCs cultured in hypoxia (1% $O_2$), when injected intramuscularly in SCID mice, showed better survival and retention *in vivo* than cells maintained in normoxic conditions.[93] In another study, hypoxia-grown MSCs were intratracheally instilled into mice with bleomycin-induced pulmonary fibrosis. Histopathological examination showed a high number of the primed MSCs in the mouse lungs at day 21, while normoxic controls were not detected.[94] Likewise, hypoxia pre-conditioned MSCs, intranasally delivered to the brain in a mouse intracerebral hemorrhagic stroke model, successfully migrated to peri-wound regions and secreted growth factors to increase neurogenesis.[95]

However, there has not been a consensus on the optimal hypoxic conditions for MSC pre-conditioning, as different studies employ varying levels and durations of oxygen depletion and utilise MSCs from different tissue and donor sources. Optimising hypoxic priming conditions is important, as different extents of oxygen depletion induce distinct cell responses. For example, severe hypoxia (<1% $O_2$) activates a quiescent-like state that relies on anaerobic glycolysis;[96] 1% $O_2$ levels increase MSC proliferative lifespan and reduce susceptibility to genetic damage;[97] whereas 2–5% $O_2$ levels stimulate MSC viability, stemness and proliferation.[98–100] These results are improved by a shorter (24 hours) duration of hypoxia exposure compared to a longer (72 hours) duration.[100]

In addition to hypoxia, serum deprivation has been shown to significantly enhance expression of pro-survival and pro-angiogenic factors such as VEGF, ANGPT, IGF and HGF. MSCs cultured in serum-deprived conditions exhibit a subpopulation of cells that maintains a longer telomere length, displays higher expression of the pluripotency transcriptional factor Oct-4, and proliferates at a higher rate than MSCs cultured with serum.[101] Furthermore, serum-deprived MSCs demonstrate significantly higher angiogenic potential in a chorioallantoic membrane assay than control MSCs.[102] Similarly, serum-free grown MSCs show improved lung engraftment, increased IL-6 levels, increased regulatory T cells in circulation, and enhanced antifibrotic effects, suppressing bleomycin-induced inflammation in a pulmonary inflammation model.[103] In an acute colitis model, umbilical cord MSCs grown without serum demonstrate improved migration to the injured colon, and dampened inflammation by promoting polarization of intestinal macrophages to produce anti-inflammatory cytokines (IL-10) and lower their secretion of inflammatory signals (TNFα), compared to serum-grown MSCs.[104]

Reactive oxygen species (ROS)-induced oxidative stress is one of the main causes of MSC death following engraftment. However, studies have demonstrated that exposure to low levels of ROS protects cells against subsequent oxidative stress.[105,106] MSCs pre-conditioned with $H_2O_2$ showed decreased cell death, increased expression of phosphorylated Akt-1, and upregulated HIF1α *in vitro* compared to unprimed MSCs, which may translate to better survival after transplantation.[107] In a rodent wound healing model, $H_2O_2$ pre-conditioned MSCs not only improved wound healing (characterised by increased microvessel density and more rapid wound closure), but also demonstrated enhanced proliferation, migration and survival in the wound site compared to non-treated MSCs.[108] In a mouse model of idiopathic pulmonary fibrosis, intratracheal instillation of umbilical cord vein MSCs pre-conditioned with $H_2O_2$ demonstrated increased survival, proliferation and grafting rates, along with increased alveolar space and reduced collagen deposition.[109] While exposure to oxidative stress is effective in enhancing MSC post-transplantation retention and function, it is a dual-edged sword, and exploring optimal ROS concentrations for cell preconditioning is a pre-requisite for therapeutic applications.

In summary, pre-conditioning of MSCs is a highly feasible clinical option as the adaptability of several priming methods, like hypoxia, can easily be integrated into a clinical pipeline. Some growth factors like GCSF have already been used in clinical trials, and a combinatorial approach with other biomolecules may improve priming efficacy and the persistence of functional benefits.

### Surface modification of MSCs

Another method to enhance the homing ability of human MSCs is *via* modification of the cell surface to increase cell recognition of homing and engraftment signals. Surface modification can be performed with natural or synthetic polymers. These polymers interact with the cell surface either by covalent conjugation, hydrophobic interaction, and/or electrostatic interaction, and allow subsequent attachment of functional moieties.[110] Studies have shown that the capacity of MSCs for homing decreases with *ex vivo* passaging, compared to primary MSCs.[111] Surface modification of MSCs with lipid-PEG and recombinant CXCR4 is shown to enhance the cell migratory response to an SDF-1 gradient by two-fold *in vitro*.[110] In another example, Sarkar and co-workers developed a biotinylated cell rolling ligand, Sialyl Lewis$^x$ (SLeX), and conjugated it to the MSC surface. The engineered SLeX-MSCs demonstrated a rolling response on a P-selectin coated substrate *in vitro*.[112] In a mice model of inflammatory bowel disease, MSCs coated with antibodies against addressins showed enhanced delivery to the colon and increased therapeutic effects. Likewise, VCAM antibody-coated MSCs demonstrated higher homing to the mesenteric lymph node and colon, and improved survival rates when compared to non-coated MSCs.[113]

Cell surface modifications offer a functionally-targeted and potentially reversible approach to modulate cellular homing.[114,115] However, more *in vivo* studies need to be performed to validate several fundamental principles of biocompatibility, such as cell viability, interference of the surface coating with the diffusion of molecules across membranes, or disruption of growth factor-ligand interactions that may be important for cellular homeostasis and membrane flexibility.[115] To allow broader clinical translation, the cost of surface engineering for therapeutic applications must also be affordable.

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### Genetic modification of MSCs

Genetic engineering provides a valuable tool to alter the DNA of an organism to obtain a desired phenotype. This can be achieved *via* various methods such as viral transduction, nuclear transfer or transfection.[116] Genetic modification of MSCs can enhance the expression of specific genes that promote homing and engraftment. Like the priming methods discussed above, genetic modification was first attempted to increase the expression of the chemokine receptor CXCR4. Transduced MSCs with elevated CXCR4 levels show increased migration towards the SDF-1 chemokine gradient, and improved homing to the infarcted myocardium compared to non-transduced MSCs.[117,118] Similarly, CXCR4-overexpressing bone marrow-derived rat MSCs exhibit enhanced chemotactic and paracrine characteristics. Injecting these MSCs into an LPS-induced acute lung injury model improves homing in target tissues and suppresses inflammatory molecules.[119]

Various iron-based magnetic nanoparticles that actively augment CXCR4 expression in MSCs have also been tested in brain injury models.[120] MSCs were non-virally transfected with magnetosome-like ferrimagnetic iron oxide nanochains (MFIONs) to overexpress brain-derived neurotrophic factor (BDNF) for stroke treatment in a rodent model. Interestingly, internalization of MFIONs promoted the homing of MSCs to the ischemic cerebrum by upregulating CXCR4 levels.[121] In addition to CXCR4, the chemokine receptor CCR1 is also known to be important for engraftment and survival. Overexpression of CCR1 in MSCs protects from serum deprivation-induced apoptosis *in vitro* and increases cell accumulation in an infarcted murine myocardium.[122]

Similarly, genetic overexpression of growth factors can improve targeted MSC migration and retention. Human pigment epithelium-derived growth factor (PEDF) overexpression *via* lentiviral delivery in MSCs increases MSC localization towards hepatocellular carcinoma in both *in vivo* and *in vitro* migration assays. Furthermore, homing of PEDF-transduced MSCs to primary tumors suppresses tumor growth and metastasis, resulting in better therapeutic outcomes.[123] Chen and coworkers (2021) transplanted adenovirally-transduced $VEGF_{165}$-expressing MSCs in a rat model of acute liver failure, and reported enhanced multipotency and increased homing and colonization of cells in liver tissue.[124] Likewise, MSCs, which have been modified to express growth differentiation factor 11 (GDF11) and maintained in hypoxia, display reduced cellular apoptosis, increased paracrine effects and preserved mitochondrial function. When transplanted into infarcted mouse hearts, these cells survive and are retained in the peri-infarct regions, resulting in increased angiogenesis, reduced scar size, and improved cardiac function.[125] Similar results are observed with MSCs overexpressing FGF2. Upon transplantation to infarcted myocardium, these cells exhibit increased viability in host tissue.[126]

To increase MSC survival post-transplantation, genetic approaches have also targeted various signaling mediators of cell survival or proliferation pathways like Akt1, a serine/threonine kinase known for its anti-apoptotic effects against oxidative or osmotic stress, irradiation, and ischemic shock.[127] Retroviral transduction of Akt1 in mouse MSCs reduces apoptosis, and improves homing and survival until 14 days post-transplantation in a GvHD model. Akt1 expression in MSCs additionally increases their immunomodulatory capacity *via* IL10 secretion, resulting in decreased levels of inflammatory cytokines TNFα and IFNγ, and attenuated liver injury.[128] In further support of this strategy, transplantation of Akt1-transduced MSCs in ischemic rat heart significantly reduces remodelling, regenerates 80–90% of lost myocardial volume, and normalizes cardiac function. These positive outcomes are proposed to be due to the enhanced retention and myocyte differentiation of Akt-MSCs in the infarcted myocardium.[129]

MSCs transfected to express Bcl-2, a regulator of apoptosis, display 32% decreased apoptosis after transplantation to a myocardial infarct region. This improved survival is associated with therapeutic benefits, including increased ventricular function, VEGF secretion, increased capillary density in the peri-infarct region, and reduced infarct size.[130] MSCs overexpressing both Bcl-2 and VEGF demonstrate increased proliferation and paracrine effects, and reduced apoptosis and autophagy, compared to cells transduced with a single gene construct.[131] In another study, MSCs overexpressing protein kinase C epsilon (PKCε), a regulator of cell apoptosis and survival, are shown to persist and be retained post-transplantation, resulting in improved cardiac function and remodelling.[132]

Post-transplantation survival of MSCs can also be enhanced by engineering the cells to increase expression of cytoprotective or stress response proteins. For example, lentiviral gene delivery of heat shock protein 70 (Hsp70) increases MSC survival and resistance to cell death under conditions of hypoxia and ischemia.[133] MSCs overexpressing Hsp20 escape oxidative stress-induced apoptosis *in vitro* and accordingly, show two-fold increased survival after transplantation, contributing to increased angiogenesis and reduced fibrosis in a rat cardiac infarct model.[134] Similarly, MSCs transfected with plasmids bearing the cytoprotective HO-1 gene show significantly reduced apoptosis in an ischemic heart. Seven days post-transplantation, these MSCs show five-fold increased cell survival compared to control cells.[135] HO-1-overexpressing MSCs also show promise in an ischemia/reperfusion rat model of acute kidney failure, by decreasing tubular necrosis and improving kidney function compared to controls. These improved therapeutic benefits are attributed to the increased cell retention of HO-1 bearing MSCs.[136]

MSCs can also be modified with non-coding microRNA to enhance post-delivery outcomes. MSC transfection with microRNA-378 enhances cell survival and vascularization potential under hypoxic conditions *in vitro*, as marked by increased proliferation, development of more extensive vascular branches, increased VEGF, PDGF, and TGFβ levels, and decreased TNFα levels.[137]

More recently, MSCs can also be modified to deliver biologic drugs like Etanercept, which is a fusion protein combin-

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ing soluble TNF receptor 2 with the Fc domain of IgG. Etanercept alleviates elevated TNF levels in patients with rheumatoid arthritis. Transduced MSCs locally injected in a mouse collagen-induced arthritis model reduce joint inflammation and promote joint repair. These benefits are proposed to be due to the enhanced cell homing, engraftment, and survival until 14 days post-injection. Furthermore, transduced MSCs possess a longer life-span and stronger chondrogenic potential compared to naïve MSCs.[138]

While genetic modification shows promise as a means of improving MSC delivery, retention and survival, such approaches are accompanied by safety concerns, such as tumorigenic risks or potential host integration of viral DNA.[56] To address these concerns, MSCs can be transfected with non-viral agents such as mRNA.[139] Cells engineered with a triple construct of P-selectin glycoprotein ligand-1, Sialyl-Lewis X, and interleukin 10 display better homing to the inflamed spinal cord in a multiple sclerosis model, driving microvasculature formation, protection from CD4+ T lymphocytes, and improved myelination.[140] However, the effects of mRNA transfections are transient, and transfection efficiencies are relatively lower compared to viral methods.[141]

In summary, commercial advancements in various engineered cell therapies have made genetic modifications in MSCs feasible for clinical use. However, approval for clinical application and large-scale manufacturing could make these therapies expensive (USD 30–100 K per treatment),[55] which can significantly hamper their accessibility in clinical practice.

## Biomaterials for MSC transplantation

Biomaterial-assisted or biomaterial-based cell therapies hold great promise, as biomaterials serve as a platform to enhance cell delivery, engraftment, and survival during and after implantation (Fig. 4). Moreover, biomaterials offer the ability to couple the physical environment of cells with chemical and biological agents that can additionally determine cell fate. Researchers have employed a range of naturally derived and synthetic materials as biomaterials. Natural materials, such as alginate,[142] chitosan, dextran,[143] gelatin,[144] hyaluronic acid,[145–147] silk fibroin,[148–150] elastin[151] and its monomer tropoelastin[152,153] are favoured due to their natural cell recognition sites and biocompatibility. However, natural biomaterials often exhibit poor mechanical strength, rapid degradation, batch-to-batch variation, and limited availability.[154] Synthetic biomaterials, such as polyethylene glycol (PEG),[155] polycarbonate (PC), polyurethane (PU),[156] poly(lactic-*co*-glycolic acid) (PLGA),[145] and polycaprolactone (PCL),[157] offer tuneable mechanical properties and low immunogenicity upon transplantation.[158] The lack of cell recognition sites on synthetic materials can be addressed through biofunctionalisation, which involves decorating bioinert surfaces with binding sites for cytokines, growth factors, and peptides. Functional moieties can be non-covalently and/or covalently immobilised on biomaterial surfaces, *via* chemical, enzymatic, and/or plasma surface modifications,[159] to facilitate cell-material integration or to instruct cell responses.

Material properties, such as biocompatibility, bioactivity, biodegradability, and mechanical properties, determine the suitability of a biomaterial as a stem cell delivery platform.[160] In the context of biocompatibility, parameters such as cytotoxicity, sensitisation, hemocompatibility, pyrogenicity, genotoxicity, and carcinogenicity are among the many tests that are performed whenever a material is intended to be implanted alongside cells.[161] Administered biomaterials must be degradable through natural metabolic pathways, and the by-product must not be toxic.[162] Mechanical properties such as topography, stiffness, and elasticity can be tailored to guide cell migration, tissue regeneration, and vascularisation.[163] The

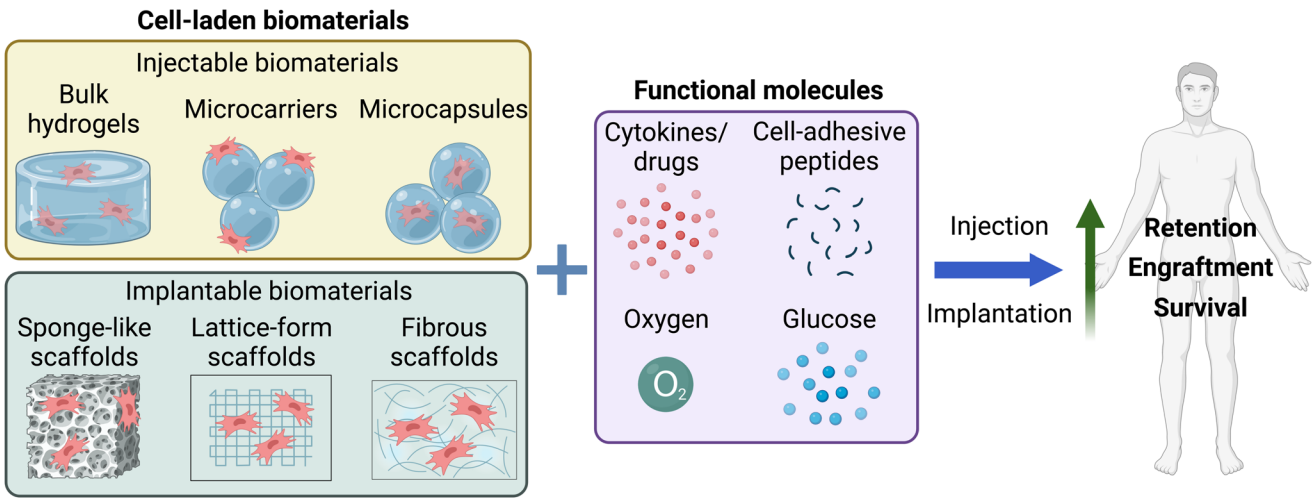


**Fig. 4** Biomaterial-assisted cell therapies utilise injectable and implantable biomaterials to improve stem cell transplantation. Injectable biomaterials, such as bulk hydrogels, microcarriers, or microcapsules, and implantable scaffolds with sponge-like, lattice-form, or fibrous compositions, can be functionalised with bioactive molecules to achieve better cell delivery, engraftment, retention, and survival for improved clinical outcomes.

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design of material biomechanical properties to instruct stem cell fate has been comprehensively reviewed elsewhere.[164]

Materials for MSC delivery can be classified into two major categories: injectable and implantable biomaterials.[160] Each method of delivery has its advantages and disadvantages, and both have been explored to repair a wide range of tissues including heart, cartilage, bone, and tendon.[165–168] For example, in the treatment of myocardial infarction, injectable biomaterials are more commonly used to deliver MSCs to the intramyocardial space due to the relative accessibility of this approach. Alternatively, implantable cardiac patches can also be applied to deliver MSCs proximally to injured tissue. Similarly, implantation of a heart pouch allows the repeated administration of MSCs, without repeated invasive open chest surgeries.[156,169] Both injectable and implantable MSC-laden biomaterials have been extensively investigated for MSC delivery, and some of these advances are highlighted in the next sections.

### Injectable MSC-encapsulating biomaterials

Injectable biomaterials can be fabricated using various methods, such as Diels–Alder reaction, Schiff base reaction, photo-crosslinking, electrostatic crosslinking, and click chemistry.[170–172] Injectable hydrogels are utilised as a cell carrier to help retain injected cells and provide a microenvironment that enhances cell viability and function. These hydrogels can take the form of a bulk material with suspended MSCs or MSC aggregates, microcarriers with MSCs seeded on the surface, microcapsules with encapsulated MSCs, or as a combination of these forms. Bulk hydrogels are the simplest injectable materials in terms of material preparation, cell encapsulation, and administration,[173] but may possess limited nutrient and oxygen diffusion relative to hydrogel density and dimensions.[174] Encapsulation of MSCs within a bulk hydrogel poses an upper limit for the dimension and size of the injected biomaterial. When the distance between cells and blood vessels is more than ~150–200 μm, necrosis is observed due to the limited nutrient and oxygen diffusion.[175,176] Additionally, bulk hydrogels are not beneficial for cell infiltration, which is crucial for tissue regeneration, due to the lack of microporosity. To improve material porosity while retaining ease of manufacturing, granular hydrogels or microgels were developed. Muir *et al.* has demonstrated the injectability and mechanical properties of photo-crosslinkable norbornene-modified hyaluronic acid-based granular hydrogels fabricated through extrusion fragmentation.[177] Multicellular spheroids of HUVECs and MSCs in a 2 : 1 ratio were mixed and co-injected with the granular hydrogels, and cytocompatibility was evidenced by cellular outgrowth after three days.[178] Although additional cell studies are required to demonstrate the use of granular hydrogels for MSC administration, this advancement holds promise for bulk hydrogels as an injectable cell delivery vehicle.

As alternatives to bulk hydrogels, microcarriers provide interconnected pores that aid cell migration and interaction,[179] while microcapsules provide customisable encapsulation and protection for individual cells.[180] Microcarriers and microcapsules have natural void space between the cell-laden pockets, which allow nutrient diffusion and cell ingrowth. However, both approaches require lengthy processing steps before transplantation, such as microcarrier manufacturing, cell seeding and encapsulation. In response, advancements have been made using high throughput microfluidics to generate microcarriers and microcapsules in a rapid and scalable manner.[181,182]

Injectable biomaterials are commonly used for stem cell delivery due to their relative ease of administration. Hydrogels are particularly advantageous in retaining cells at the site of implantation, due to their viscous, Newtonian fluid nature and the ability to crosslink the material either *ex situ* or *in situ* after injection.[183] Increasing hydrogel viscosity prolongs the time that cells are localised at the desired location, and reduces the risk of cells being removed before they can attach to the target site.[184] It is essential that crosslinking parameters, such as the chemical initiator, pH, temperature, ion concentration, and wavelength, are well-tolerated by the encapsulated cells. Additionally, the hydrogel matrix provides a high surface area for cell interaction, while facilitating the diffusion of nutrients and waste.

Another critical cytoprotective feature of injectable hydrogels is their shear-thinning property. When MSC suspensions are directly injected through a needle, cells experience mechanical stresses, such as shear or extensional stress, due to the frictional force exerted parallel to the needle wall during liquid ejection. Using a narrow needle bore size increases the apoptosis of ejected cells,[185] which can be ameliorated by a slower flow rate.[186] Encapsulating MSCs within a hydrogel solution mimics the effects of a slower flow rate. When MSCs are suspended in saline water, injecting the cells through a larger needle bore size (26G) and with a slow flow rate (1 μL $min^{-1}$) reduces cell death by half, compared to using a small needle bore size (32G) and high flow rate (10 μL $min^{-1}$). In contrast, when MSCs are suspended in a more viscous pluronic hydrogel, the increase in cell death associated with a high flow rate is blunted.[185] Other shear-thinning hydrogels fabricated from alginate[187,188] and collagen[189] have similarly improved cell delivery and retention at the implant site.

Cai *et al.* showcased a double crosslinked hydrogel system termed shear-thinning hydrogel for injectable encapsulation and long-term delivery (SHIELD). The SHIELD construct protects cells during injection and improves retention at the site of implantation. These dual effects are achieved by first encapsulating adipose derived MSCs within the hydrogel *via* peptide-based crosslinking, followed by *in situ* thermal transition of poly(*N*-isopropylacrylamide) (PNIPAM) chains with PEG after injection. The first crosslinking step provides cells with mechanical protection, resulting in 93% post-injection cell viability, compared to 69% when cells are co-injected with saline. The secondary *in situ* crosslinking step enhances cell retention, with 60% of hydrogel-encapsulated cells remaining viable cells at day 3, in contrast to 13% of control cells co-injected with phosphate-buffered saline (PBS). These benefits

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persist until day 14, cumulating in a six-fold increase in cell retention over control samples.[190]

Similarly, Lee *et al.* reported on cell viability and retention within an injectable thiolated hyaluronic acid hydrogel, when administered in a mouse model of atopic dermatitis. Subcutaneous application of MSCs within the hydrogel increases cell detection by 50-fold, compared to cells in PBS, at three weeks post-injection. Hydrogel encapsulation of the MSCs is also associated with improved immunomodulation capability, leading to reduced epidermal thickness and mast cell infiltration, and a lower expression of major inflammatory cytokines such as IL13, C–C motif chemokine ligand 11 (CCL11), and CCL24.[173]

In addition to cell retention, cell survival at the implant site is another key consideration for successful cell therapy. MSC-laden hydrogels are often transplanted to injured sites that are typically inflamed, hypoxic, nutrient-poor, and lacking in cell binding signals. To offer additional protection against cellular stresses at the implant site, hydrogels can be designed to incorporate functional groups such as cell-binding motifs and antioxidative agents.[191] Crosslinking hydrogels with extracellular matrix-derived cell-adhesive motifs, such as the Arg-Gly-Asp (RGD) peptide derived from fibronectin,[192] the Ile-Lys-Val-Ala-Val (IKVAV) peptide derived from laminin,[193] and/or collagen binding domains,[194] has been shown to improve MSC retention post-transplantation. For instance, at 28 days post-injection, stem cells loaded within a self-assembling peptide amphiphile gel functionalised with the IKVAV epitope are retained in significantly higher numbers than control cells in DMEM.[195] As another example, cross-linking hydrogels with ROS-degradable polymers is shown to improve cell viability up to 40%, compared to conventional MMP-degradable hydrogels. Martin *et al.* suggested that MMP production at pathological sites is highly variable; therefore, by harnessing the presence of ROS instead, a more stable and antioxidative environment can be constructed for improved cell viability.[155]

To combat the issue of MSC clearance by immune cells, immunomodulatory factors such as cytokines and drugs can be used in conjunction with cell-laden hydrogels.[196–198] For instance, after immobilising IGF1 into a chitosan-based hydrogel, the encapsulated MSCs show enhanced proliferation, retention at the site of implantation, and improved clinical outcomes in a mouse colitis model. It was hypothesised that this therapeutic improvement is due to the elevated secretion of PGE2 by MSCs, which, with IL10, mediates polarisation towards M2 macrophages, thus lowering the immune response and improving cell survival.[196] Another approach immobilises the anti-inflammatory drug, infliximab, in hydrogels, which suppresses TNFα activity. This antibody-functionalised hydrogel improves MSC proliferation, differentiation and extracellular matrix production *in vitro*. Upon implantation into a rabbit rheumatoid arthritis model, the cell-laden material suppresses inflammatory cytokine levels, and improves cartilage and subchondral bone repair.[198] Similarly, an antioxidative drug, tempol, has been conjugated onto nanoparticles and encapsulated in a hydrogel. When co-delivered with ectomesenchymal stem cells in a rat inflammatory periodontitis model, the functionalised construct attenuates oxidative and inflammatory responses, which correlates with increased cell survival and osteogenic differentiation.[197]

Injured or disease sites are often nutrient- and oxygen-deprived, which is challenging for cell survival and maintenance. The "dying stem cell" hypothesis postulates that transplanted MSCs are removed by innate and adaptive immune responses.[199] Recently, it was demonstrated that the short lifespan of transplanted MSCs is due to the activation of cellular hypoxia signalling pathways, followed by caspase 3-mediated apoptosis, which leads to local recruitment of immune cells, and eventual engulfment and clearance by macrophages.[200] Therefore, the survival of transplanted MSCs may depend on the extent of local oxygenation. To facilitate long-term cell survival, cells should be supplied with oxygen until vascularisation occurs. Oxygen- and nutrient-carrying hydrogels have been designed to maintain a favourable environment for transplanted MSCs. Different approaches of short- and long-term oxygenation exist to increase stem cell survival and therapeutic efficacy, such as the use of hyperbaric oxygenation to systemically deliver oxygen;[201] *in situ* oxygen generation with $MgO_2$,[202] $CaO_2$,[203] and $H_2O_2$;[204,205] and material functionalisation with oxygen carriers, such as hemoglobin,[206] myoglobin,[207] and fluorinated compounds such as perfluorocarbons (PFC).[208] Niu *et al.* have reported that under 1% $O_2$ condition, MSCs encapsulated in a PFC-carrying hydrogel show significantly improved cell survival and proliferation over unprotected MSCs for 14 days, further highlighting the importance of oxygenation during biomaterial-based stem cell transplantation.[208]

The lack of glucose, a primary source of cellular energy, is another major factor that hampers MSC survival after transplantation.[209,210] Several studies have examined the benefits of locally supplying glucose for MSC maintenance. For example, MSCs incubated with glucose-carrying microspheres survive better within the first 36 hours post-implantation, compared to cells in PBS, or cells incubated with free glucose.[210] To prolong cell survival, a slow glucose-releasing hydrogel has been developed based on the laminaran glucan. The hydrogel is designed to be degradable by the Bgl1B enzyme, which hydrolyses laminaran into glucose. Over 14 days, the hydrogel is shown to improve *in vitro* and *in vivo* cell survival when compared to no-enzyme controls.[211]

The benefits of injectable biomaterial-based MSC delivery platforms are attributed to several factors. Firstly, their injectability and ease of handling make them an attractive option for minimally invasive implantation, which is favourable for clinical applications to reduce surgery costs and enable repeated administration. Secondly, the hydrogels serve as a platform that can incorporate dynamic physical, chemical and biological cues to support encapsulated cells and instruct resident cells at the injection site. By incorporating nutrients, oxygen, growth factors, and drugs into the hydrogel, stress-induced cell death is reduced, and local cell retention and survival are enhanced.[147,173] Due to their injectability, such bio-

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materials usually possess lower mechanical strength, which is useful for soft tissue repair. However, despite encouraging results in many preclinical and some early clinical studies, therapeutic outcomes can vary between individuals. Therefore, future approaches should explore the combinational use of multiple biomaterials, functional molecules and cell types, to help achieve an integrated stem cell niche for long-term dynamic modulation of intracellular and extracellular responses throughout the process of tissue regeneration, and to suit different host environments and a broad range of clinical applications.

A summary of notable advances in the design and use of injectable hydrogels for improved MSC delivery, retention, and survival is outlined in Table 2.

### Implantable MSC-laden biomaterials

Stem cell transplantation *via* implantable biomaterials typically requires more invasive procedures such as open chest or knee surgeries.[217,218] Additionally, if MSC re-implantation is required, additional surgery may not be immediately feasible. Nevertheless, this approach to stem cell transplantation harbours promise due to the potential of macroporous scaffolds to mimic the native MSC microenvironment. Such scaffolds can possess more suitable biomechanical properties over injectable systems, with tailorable stiffness, pore size, and topography, which can enhance tissue integration and facilitate the formation of complex tissue architecture.[219] Pre-seeding MSCs on polymer surfaces also reduces the risk of anoikis, or cell death triggered by the detachment of anchorage-dependent cells from the extracellular matrix.[220] Macroporous scaffolds are commonly fabricated using synthetic polymeric substrates through various methods such as solvent casting, electrospinning, gas foaming, freeze-drying, particle leaching, laser sintering, photolithography, stereolithography, and additive manufacturing.[172,221]

Sponge-like, fibrous, and lattice-form scaffolds have been extensively tested as cell delivery vehicles in pre-clinical and clinical settings.[219,222] The large, interconnected pores of these scaffolds offer advantages in efficient nutrient and waste diffusion, as well as cell migration and tissue integration. Both natural and synthetic materials are used to fabricate porous scaffolds, while additional steps of surface modification and functionalization may be required for synthetic materials. As conventional surface modification techniques such as plasma cleaning are limited to thin planar films, three-dimensional scaffolds are often pre-soaked with serum-containing media for extended periods of time, with the aim of coating the scaffold surfaces with cell-binding proteins. However, such physisorbed proteins can subsequently be replaced by other molecules with a higher affinity to the surface, which can lead to undesired cell detachment from the scaffold after implantation.[223] To enable covalent anchorage of biomolecules across 3D scaffolds, recent advances include additive manufacturing coupled with plasma treatment technology in a layer-by-layer fashion to print 3D porous scaffolds with covalent binding capability.[224] Porous scaffolds are also shown to be surface modified across the scaffold thickness using packed-bed plasma ion implantation.[225,226] Surface modification enables scaffolds to be functionalised with cell-instructive biomolecules such as extracellular matrix proteins, peptide motifs, cytokines, or growth factors. Matrix proteins, such as collagen, fibronectin, laminin, elastin, or vitronectin, and peptide derivatives, have been used to enhance the biocompatibility of implantable materials.[159,227,228] Growth factors, such as VEGF, FGF2, EGF, TGFβ, or PDGF have been used to promote cell proliferation, wound healing and tissue morphogenesis.[159]

The benefits of stem cell delivery *via* implantable biomaterials are widely showcased in literature (Table 3). Zamproni *et al.* demonstrated in a mouse stroke model that porous PLA scaffolds support enhanced MSC delivery and retention at the injury site, thus significantly reducing lesion area, compared to direct intracerebral injection of cells. Increased local cell retention is potentially attributed to the increased integrin α6 and CXCL12 secretion by MSCs on the scaffolds compared to those on flat coverslips.[229] As another example, poly-*p*-xylylene porous scaffolds fabricated using ice templating and functionalised with FGF2 and L-ascorbic acid 2-phosphate are shown to enhance MSC proliferation, renewal, differentiation, and *in vivo* outcomes, including blood vessel ingrowth and induction of osteoblast growth enhancing osteointegration upon implantation in a rat calvarial bone defect, compared to scaffolds lacking one or both bioactive molecules.[230] Similar to injectable materials that increase local oxygen supply for administered MSCs, 3D printed PCL and nanohydroxyapatite scaffolds can also be oxygenated using gelatin-$CaO_2$ microspheres mixed with the polymer filaments. Compared to non-oxygenated scaffolds, the oxygenated scaffolds exhibit favourable mechanical properties, promote MSC survival in hypoxic conditions, and enhance MSC proliferation and osteogenic differentiation, resulting in enhanced bone regeneration at 4 and 12 weeks after implantation in a rabbit cranial defect.[231]

A major therapeutic mechanism of MSCs is their secretion of extracellular vesicles (EV), which carry a range of intercellular signalling factors. Scaffolds can be designed to not only improve cell retention and survival, but also promote EV secretion. For example, MSCs can be encapsulated within a PLA/PLLA/PEG-based porous scaffold, which allows exosome release through the scaffold pores. The scaffold is shown to support increased EV secretion, cell retention and survival upon implantation in a myocardial infraction model, compared to direct injection of MSCs.[235] Additionally, to bypass the need for and challenges of MSC reimplantation into organs such as heart, Mei *et al.* have developed a similar strategy to harness the paracrine effects of MSCs, while protecting the cells with a scaffold. Their design of an origami-shaped heart pouch allows multiple reinjections of MSCs after the initial device implantation. The cell-containing pouch is made of thermoplastic PU or polyethylene and PC, while a nylon semi-permeable membrane allows the diffusion of EVs to neighbouring diseased tissue. MSCs encapsulated in the pouch display increased viability and enhanced VEGF, HGF,

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**Table 2** Injectable biomaterial-based platforms for MSC delivery

| Biomaterial | MSC origin | Cell incorporation | Benefits and outcomes | Animal model | Ref. |
|---|---|---|---|---|---|
| Gelatin methacrylate and oxidised dextran composite hydrogel functionalised with graphene oxide | Human umbilical cord | MSCs encapsulated in bulk hydrogel | Enhanced cell retention, improved differentiation potential, reduced infarct size and cardiac fibrosis in infarct zone, increased ventricular ejection fraction | Rat (heart) | 212 |
| Hyaluronic acid hydrogel containing human VE-cadherin coated poly (lactic-*co*-glycolic acid) (PLGA) microparticles | Human | MSC aggregates encapsulated in hydrogel | Decreased expression of inflammatory cytokines, increased expression of angiogenic factors, reconstruction of rat cardiac function, structure, and revascularisation | Rat (heart) | 145 |
| Naphthalene modified hydrogel with glycine–phenylalanine–phenylalanine–tyrosine–glutamic acid and linked with two glutamic acids | Human placenta | MSCs encapsulated in bulk hydrogel | Increased cell viability, enhanced pro-angiogenic and anti-apoptotic effects, improved *in vivo* cell retention and blood perfusion | Mouse (intramuscular) | 213 |
| Polyethylene glycol (PEG) hydrogels crosslinked with acrylated heparin | Rat bone marrow | MSCs encapsulated in bulk hydrogel | Improved MSC retention at injection site, reduced ventricular remodelling, stimulation of neo-vasculogenesis, increased secretion of pro-angiogenic factors | Rat (heart) | 169 |
| Growth factor reduced Matrigel | Human umbilical cord | MSCs crosslinked with microspheres in hydrogel | Increased and prolonged retention until day 35, increased endometrium thickness and fertility | Rat (endometrium) | 214 |
| Hydroactive gel (187990, CONVATEC, USA) | Human Wharton's jelly | MSCs encapsulated in bulk hydrogel | Prolonged cell retention, improved diabetic wound healing and regeneration, increased M1 to M2 macrophage transformation, increased cell proliferation, neovascularisation at wound site | Rat (intraperitoneal) | 146 |
| Thiol-functionalised hyaluronic acid | Human adipose | MSCs encapsulated in bulk hydrogel | High cell viability, good biocompatibility, increased expression of anti-inflammatory cytokines, reduced thickness of epidermis and mast cell infiltration | Mouse (subcutaneous) | 173 |
| PEG macromers and thiolated hyaluronic acid | Adipose | MSCs encapsulated in bulk hydrogel | Good mechanical properties, improved cell retention at site of injection, improved healing, inhibited inflammation, enhanced angiogenesis and re-epithelialisation | Mouse (subcutaneous) | 147 |
| Gelatin microcarriers | Human umbilical cord | MSCs seeded on microcarrier | Reduced dosage of MSCs, decreased levels of inflammatory factors, accelerated chondrogenesis, increased extracellular matrix interaction and phenotypic maintenance of chondrocytes | Rat (subcutaneous) | 144 |
| Alginate hydrogel | Human bone marrow | MSCs encapsulated in hydrogel microspheres | Increased cell viability, enhanced immunomodulatory effects, reduced cartilage degeneration | Rat (intraarticular) | 142 |
| Graphene oxide, poly(*N*-isopropylacrylamid) and gelatin methacrylate microcarriers loaded with lipopolysaccharide | Not specified | MSCs seeded on microcarrier | Improved MSC retention over 7 days, improved anti-inflammatory ability, improved liver regeneration. | Rat (intraperitoneal) | 215 |
| Polyethylene glycol diacrylate and poly(vinyl alcohol) hydrogel microcapsules | Human umbilical cord blood | MSCs encapsulated in microcapsules | Enhanced MSC retention and survival, localised tissue repair, reduced colonic macrophage infiltration, reduced severity of irritable bowel disease | Mouse (oral to colon) | 180 |
| Hyaluronic acid core and alginate shell microcapsules | Human umbilical cord | MSCs encapsulated by microcapsules | Improved MSC survival against oxidative and shear stress, enhanced growth factor secretion, increased angiogenesis | Mouse (subcutaneous) | 216 |
| Polyethylene glycol hydrogels crosslinked with reactive oxygen species-degradable poly(thioketal) polymers | Mouse bone marrow | MSCs encapsulated in hydrogel | Increased cell viability and retention | Mouse (subcutaneous) | 155 |
| Polyethylene glycol microgels coated with FXIII or thrombin | Mouse | MSCs co-injected with microgel | Enhanced MSC retention, proliferation and survival, tissue ingrowth and vascularisation | Mouse (subcutaneous) | 179 |

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Table 3 Implantable biomaterials for MSC delivery

| Biomaterial | MSC origin | Cell incorporation | Benefits and outcomes | Model | Ref. |
|---|---|---|---|---|---|
| Heart pouch made of thermoplastic polyurethane (TPU) film on top, and nylon semi-permeable membrane at bottom, separated by a TPU origami lattice (rodent study) or polyethylene film (pig study) | Human bone marrow | MSCs injected and encapsulated in pouch | Improved cell retention compared to direct cell injection, improved paracrine function, increased myocardium thickness, reduced infarct size, and increased viable cardiac tissue | Rat and pig (heart) | 156 |
| Poly(L-lactic acid-ε-caprolactone) and hydroxyapatite nanoparticle scaffold | Rat bone marrow | MSCs seeded in porous scaffold | Enhanced MSC adhesion, retention, survival, and ingrowth, improved cell-scaffold interaction, supported immunomodulatory, angiogenic and osteogeneic paracrine effects, enhanced bone vascularisation | Rat (subcutaneous) | 219 |
| Bulk platelet-rich plasma hydrogels added into 3D-printed rigid poly(lactic-*co*-glycolic acid) scaffold | Rabbit bone marrow | MSCs encapsulated in hydrogel and loaded onto porous scaffold | Supported MSC survival, improved differentiation potential, increased osteochondral regeneration due to paracrine effects | Rabbit (hind limb) | 222 |
| Demineralised bone matrix coated with collagen-binding domain/IKVAV-cRGD peptide | Human bone marrow | MSCs seeded in porous scaffold | Superior MSC retention and *in vivo* osteogenesis | Mouse (bilateral femur) | 217 |
| 3D bioprinted nanocellulose or nanocellulose-collagen 1 scaffold | Human adipose tissue, bone marrow, corneal stroma | MSCs encapsulated in hydrogel | Supported *in vitro* cell survival but not *in vivo* cell post-transplantation retention | Porcine (cornea) | 232 |
| 3D bioprinted gelatin/hydroxyapatite hybrid scaffold | Human umbilical cord blood | MSCs seeded in porous scaffold | Supported *in vitro* cell adhesion, growth, chondrogenic differentiation, aided cartilage repair at 12- and 24-weeks post-implantation | Porcine (cartilage) | 233 |
| Alginate–chitosan polyelectrolyte complex scaffold | Rat bone marrow | MSCs seeded in porous scaffold | Improved cell retention, maintained cell viability, supported vascularisation and integration with surrounding muscle | Rat (intramuscular) | 234 |

and IGF1 secretion compared to control MSCs. Upon implantation of the heart pouch in rats, the protected cells were retained at higher levels compared to directly injected cells. Repeated MSC administration *via* the pouch is demonstrated to reduce infarct size, increase infarct wall thickness, and enhance viable cardiac tissue generation.[156,236]

Implantable biomaterial-based delivery of MSCs has emerged as a promising approach for cell therapy due to its advantages over injectable methods, including enhanced cell survival and reduced issues of administration and *in situ* cross-linking. However, implantable biomaterials need to deliver all required therapeutic outcomes in a single administration, since re-implantation is less feasible. Furthermore, achieving long-term tissue mimicry and modulation is a complex challenge that requires careful consideration of several factors, including stable immobilization and presentation of biomolecules on the scaffold,[237] dynamic exchange of adhered biomolecules with non-target molecules in the implant site,[223] degradation profile of scaffolds relative to tissue healing,[238] mechanical compatibility of scaffolds with native tissue, and potential biofilm formation on the implant.[239] To facilitate the clinical translation of implantable biomaterials, interdisciplinary efforts to understand the effects of material properties on cell biology are crucial to developing a long-lasting solution that addresses these challenges. For example, recent research has highlighted the impact of scaffold mechanical properties, such as stiffness, pore size,[240] and filament alignment,[241] on the modulation of host immune responses. Understanding the interplay of material properties and the function, not only of embedded MSCs, but also of cells resident in the implant site, is important to enhance the compatibility and longevity of cell-laden implantable biomaterials.

Both injectable and implantable biomaterials have been extensively investigated for MSC delivery and have shown great promise to enhance the cells' therapeutic effects. The design and fabrication of these advanced materials share similar perspectives on improving cell engraftment, retention, survival, and function at target sites. As current materials have typically aimed to facilitate these processes *via* singular mechanisms, future work should employ a multi-strategy approach to develop multi-functional constructs that holistically promote MSC post-transplantation efficiency and therapeutic responses, including paracrine instruction of resident cells or differentiation into replacement cells.

## Conclusions

Stem cell transplantation has received substantial interest, but is accompanied by reduced post-transplant survival, retention, and engraftment due to poor cell–cell or cell–ECM interactions. Improved strategies that modify internal pathways or

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provide a protective external environment can improve transplantation efficiency and promote cell-mediated tissue repair. Evidence-based studies suggest that genetic modification, cell surface engineering, or pre-conditioning of MSCs by regulating environmental signals increases the homing and engraftment capabilities of transplanted cells, with room for improvement. Biomaterial-assisted platforms, whether natural or synthetic, and crosslinked with a wide variety of bioactive moieties, provide a gradient of signalling cues that stimulate cellular behaviour *in vivo*. Furthermore, the spatiotemporal signalling generated by these bioengineered constructs, along with those from the host tissue microenvironment, can orchestrate better cell engraftment, retention, and survival, and afford transplanted cells the opportunity to exert therapeutic benefits. However, there are certain challenges that need to be addressed, such as the conversion of products from research or pre-clinical grade to clinical grade, large scale manufacturing under cGMP conditions, and the cost of these translations for clinical applications. Integrating cellular and biomaterial strategies holds promise for overcoming the current hurdles of stem cell delivery and retention, improving the efficiency of cell usage, and reducing demands on cell supply. Developing long-term, functional, and multicellular biomaterials that can effectively integrate with host tissue and guide the regenerative response is necessary for achieving success in this field.

## Conflicts of interest

The authors have no conflicts to declare.

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## References

1 A. Keating, *Curr. Opin. Hematol.*, 2006, **13**, 419–425.
2 K. Le Blanc and O. Ringden, *Biol. Blood Marrow Transplant.*, 2005, **11**, 321–334.
3 R. Margiana, A. Markov, A. O. Zekiy, M. U. Hamza, K. A. Al-Dabbagh, S. H. Al-Zubaidi, N. M. Hameed, I. Ahmad, R. Sivaraman, H. H. Kzar, M. E. Al-Gazally, Y. F. Mustafa and H. Siahmansouri, *Stem Cell Res. Ther.*, 2022, **13**, 366.
4 D. Lodi, T. Iannitti and B. Palmieri, *J. Exp. Clin. Cancer Res.*, 2011, **30**, 9.
5 A. Ebrahimi, H. Ahmadi, Z. Pourfraidon Ghasrodashti, N. Tanide, R. Shahriarirad, A. Erfani, K. Ranjbar and S. Ashkani-Esfahani, *Bosnian J. Basic Med. Sci.*, 2021, **21**, 672–701.
6 I. R. Murray, J. Chahla, M. R. Safran, A. J. Krych, D. B. F. Saris, A. I. Caplan, R. F. LaPrade and Cell Therapies Communication Expert Group, *J. Bone Joint Surg., Am. Vol.*, 2019, **101**, 904–911.
7 S. A. Rodeo, *J. Bone Joint Surg., Am. Vol.*, 2019, **101**, e47.
8 M. F. Pittenger, D. E. Discher, B. M. Peault, D. G. Phinney, J. M. Hare and A. I. Caplan, *npj Regener. Med.*, 2019, **4**, 22.
9 U. Galderisi, G. Peluso and G. Di Bernardo, *Stem Cell Rev. Rep.*, 2022, **18**, 23–36.
10 J. J. Hwang, Y. A. Rim, Y. Nam and J. H. Ju, *Front. Immunol.*, 2021, **12**, 631291.
11 M. R. Kouchakian, N. Baghban, S. F. Moniri, M. Baghban, S. Bakhshalizadeh, V. Najafzadeh, Z. Safaei, S. Izanlou, A. Khoradmehr, I. Nabipour, R. Shirazi and A. Tamadon, *Stem Cells Int.*, 2021, **2021**, 1634782.
12 W. Zakrzewski, M. Dobrzynski, M. Szymonowicz and Z. Rybak, *Stem Cell Res. Ther.*, 2019, **10**, 68.
13 D. E. Rodriguez-Fuentes, L. E. Fernandez-Garza, J. A. Samia-Meza, S. A. Barrera-Barrera, A. I. Caplan and H. A. Barrera-Saldana, *Arch. Med. Res.*, 2021, **52**, 93–101.
14 T. D. Henry, J. H. Traverse, B. L. Hammon, C. A. East, B. Bruckner, A. E. Remmers, D. Recker, D. A. Bull and A. N. Patel, *Circ. Res.*, 2014, **115**, 730–737.
15 P. K. Gupta, M. Krishna, A. Chullikana, S. Desai, R. Murugesan, S. Dutta, U. Sarkar, R. Raju, A. Dhar, R. Parakh, L. Jeyaseelan, P. Viswanathan, P. K. Vellotare, R. N. Seetharam, C. Thej, M. Rengasamy, S. Balasubramanian and A. S. Majumdar, *Stem Cells Transl. Med.*, 2017, **6**, 689–699.
16 B. T. Freeman, J. P. Jung and B. M. Ogle, *PLoS One*, 2015, **10**, e0136199.
17 E. D. Thomas, H. L. Lochte, Jr., W. C. Lu and J. W. Ferrebee, *N. Engl. J. Med.*, 1957, **257**, 491–496.
18 S. E. Haynesworth, J. Goshima, V. M. Goldberg and A. I. Caplan, *Bone*, 1992, **13**, 81–88.
19 D. Sipp, P. G. Robey and L. Turner, *Nature*, 2018, **561**, 455–457.
20 A. O. J. Fakoya, D. A. Otohinoyi, T. Marcelle and J. Yusuf, *Open Access Maced. J. Med. Sci.*, 2017, **5**, 818–824.
21 W. Shim, A. Mehta, P. Wong, T. Chua and T. H. Koh, *Cytotherapy*, 2013, **15**, 399–415.
22 W. Shim, A. Mehta, S. Y. Lim, G. Zhang, C. H. Lim, T. Chua and P. Wong, *Cardiovasc. Res.*, 2011, **89**, 20–30.
23 I. M. Barbash, P. Chouraqui, J. Baron, M. S. Feinberg, S. Etzion, A. Tessone, L. Miller, E. Guetta, D. Zipori, L. H. Kedes, R. A. Kloner and J. Leor, *Circulation*, 2003, **108**, 863–868.
24 D. Hou, E. A. Youssef, T. J. Brinton, P. Zhang, P. Rogers, E. T. Price, A. C. Yeung, B. H. Johnstone, P. G. Yock and K. L. March, *Circulation*, 2005, **112**, I150–I156.
25 T. Freyman, G. Polin, H. Osman, J. Crary, M. Lu, L. Cheng, M. Palasis and R. L. Wilensky, *Eur. Heart J.*, 2006, **27**, 1114–1122.
26 Y. Liu, Y. Dong, X. Wu, X. Xu and J. Niu, *Stem Cell Res. Ther.*, 2022, **13**, 204.

Published on 23 may 2023. Downloaded on 28.12.2024 05:50:03.

27 F. Wang, Y. Li, B. Wang, J. Li and Z. Peng, *Crit. Care*, 2023, **27**, 31.
28 J. M. Hare, J. H. Traverse, T. D. Henry, N. Dib, R. K. Strumpf, S. P. Schulman, G. Gerstenblith, A. N. DeMaria, A. E. Denktas, R. S. Gammon, J. B. Hermiller Jr., M. A. Reisman, G. L. Schaer and W. Sherman, *J. Am. Coll. Cardiol.*, 2009, **54**, 2277–2286.
29 A. Mehta and W. Shim, *Cell Transplant.*, 2013, **22**, 1–14.
30 Q. Jiang, P. Song, E. Wang, J. Li, S. Hu and H. Zhang, *J. Mol. Cell Cardiol.*, 2013, **56**, 1–7.
31 R. H. Lee, A. A. Pulin, M. J. Seo, D. J. Kota, J. Ylostalo, B. L. Larson, L. Semprun-Prieto, P. Delafontaine and D. J. Prockop, *Cell Stem Cell*, 2009, **5**, 54–63.
32 J. Gao, J. E. Dennis, R. F. Muzic, M. Lundberg and A. I. Caplan, *Cells Tissues Organs*, 2001, **169**, 12–20.
33 S. Schrepfer, T. Deuse, H. Reichenspurner, M. P. Fischbein, R. C. Robbins and M. P. Pelletier, *Transplant Proc.*, 2007, **39**, 573–576.
34 J. Nystedt, H. Anderson, J. Tikkanen, M. Pietila, T. Hirvonen, R. Takalo, A. Heiskanen, T. Satomaa, S. Natunen, S. Lehtonen, T. Hakkarainen, M. Korhonen, S. Laitinen, L. Valmu and P. Lehenkari, *Stem Cells*, 2013, **31**, 317–326.
35 U. M. Fischer, M. T. Harting, F. Jimenez, W. O. Monzon-Posadas, H. Xue, S. I. Savitz, G. A. Laine and C. S. Cox Jr., *Stem Cells Dev.*, 2009, **18**, 683–692.
36 E. G. Schmuck, J. M. Koch, J. M. Centanni, T. A. Hacker, R. K. Braun, M. Eldridge, D. J. Hei, P. Hematti and A. N. Raval, *Stem Cells Transl. Med.*, 2016, **5**, 1668–1675.
37 J. M. Karp and G. S. Leng Teo, *Cell Stem Cell*, 2009, **4**, 206–216.
38 E. C. Perin, M. Tian, F. C. Marini 3rd, G. V. Silva, Y. Zheng, F. Baimbridge, X. Quan, M. R. Fernandes, A. Gahremanpour, D. Young, V. Paolillo, U. Mukhopadhyay, A. T. Borne, R. Uthamanthil, D. Brammer, J. Jackson, W. K. Decker, A. M. Najjar, M. W. Thomas, A. Volgin, B. Rabinovich, S. Soghomonyan, H. J. Jeong, J. M. Rios, D. Steiner, S. Robinson, O. Mawlawi, T. Pan, J. Stafford, V. Kundra, C. Li, M. M. Alauddin, J. T. Willerson, E. Shpall and J. G. Gelovani, *PLoS One*, 2011, **6**, e22949.
39 L. Liao, B. Shi, H. Chang, X. Su, L. Zhang, C. Bi, Y. Shuai, X. Du, Z. Deng and Y. Jin, *Theranostics*, 2017, **7**, 106–116.
40 P. Walczak, J. Zhang, A. A. Gilad, D. A. Kedziorek, J. Ruiz-Cabello, R. G. Young, M. F. Pittenger, P. C. van Zijl, J. Huang and J. W. Bulte, *Stroke*, 2008, **39**, 1569–1574.
41 Y. Wang, J. He, X. Pei and W. Zhao, *Nephrology*, 2013, **18**, 201–208.
42 S. Wang, Y. Li, J. Zhao, J. Zhang and Y. Huang, *Biol. Blood Marrow Transplant.*, 2013, **19**, 538–546.
43 I. Rosova, M. Dao, B. Capoccia, D. Link and J. A. Nolta, *Stem Cells*, 2008, **26**, 2173–2182.
44 K. C. Wollert, G. P. Meyer, J. Lotz, S. Ringes-Lichtenberg, P. Lippolt, C. Breidenbach, S. Fichtner, T. Korte, B. Hornig, D. Messinger, L. Arseniev, B. Hertenstein, A. Ganser and H. Drexler, *Lancet*, 2004, **364**, 141–148.
45 J. Roncalli, F. Mouquet, C. Piot, J. N. Trochu, P. Le Corvoisier, Y. Neuder, T. Le Tourneau, D. Agostini, V. Gaxotte, C. Sportouch, M. Galinier, D. Crochet, E. Teiger, M. J. Richard, A. S. Polge, J. P. Beregi, A. Manrique, D. Carrie, S. Susen, B. Klein, A. Parini, G. Lamirault, P. Croisille, H. Rouard, P. Bourin, J. M. Nguyen, B. Delasalle, G. Vanzetto, E. Van Belle and P. Lemarchand, *Eur. Heart J.*, 2011, **32**, 1748–1757.
46 E. C. Perin, J. T. Willerson, C. J. Pepine, T. D. Henry, S. G. Ellis, D. X. Zhao, G. V. Silva, D. Lai, J. D. Thomas, M. W. Kronenberg, A. D. Martin, R. D. Anderson, J. H. Traverse, M. S. Penn, S. Anwaruddin, A. K. Hatzopoulos, A. P. Gee, D. A. Taylor, C. R. Cogle, D. Smith, L. Westbrook, J. Chen, E. Handberg, R. E. Olson, C. Geither, S. Bowman, J. Francescon, S. Baraniuk, L. B. Piller, L. M. Simpson, C. Loghin, D. Aguilar, S. Richman, C. Zierold, J. Bettencourt, S. L. Sayre, R. W. Vojvodic, S. I. Skarlatos, D. J. Gordon, R. F. Ebert, M. Kwak, L. A. Moye, R. D. Simari and Cardiovascular Cell Therapy Research Network, *J. Am. Med. Assoc.*, 2012, **307**, 1717–1726.
47 K. L. Ang, D. Chin, F. Leyva, P. Foley, C. Kubal, S. Chalil, L. Srinivasan, L. Bernhardt, S. Stevens, L. T. Shenje and M. Galinanes, *Nat. Clin. Pract. Cardiovasc. Med.*, 2008, **5**, 663–670.
48 A. W. Heldman, D. L. DiFede, J. E. Fishman, J. P. Zambrano, B. H. Trachtenberg, V. Karantalis, M. Mushtaq, A. R. Williams, V. Y. Suncion, I. K. McNiece, E. Ghersin, V. Soto, G. Lopera, R. Miki, H. Willens, R. Hendel, R. Mitrani, P. Pattany, G. Feigenbaum, B. Oskouei, J. Byrnes, M. H. Lowery, J. Sierra, M. V. Pujol, C. Delgado, P. J. Gonzalez, J. E. Rodriguez, L. L. Bagno, D. Rouy, P. Altman, C. W. Foo, J. da Silva, E. Anderson, R. Schwarz, A. Mendizabal and J. M. Hare, *J. Am. Med. Assoc.*, 2014, **311**, 62–73.
49 D. L. Kraitchman, M. Tatsumi, W. D. Gilson, T. Ishimori, D. Kedziorek, P. Walczak, W. P. Segars, H. H. Chen, D. Fritzges, I. Izbudak, R. G. Young, M. Marcelino, M. F. Pittenger, M. Solaiyappan, R. C. Boston, B. M. Tsui, R. L. Wahl and J. W. Bulte, *Circulation*, 2005, **112**, 1451–1461.
50 M. Agung, M. Ochi, S. Yanada, N. Adachi, Y. Izuta, T. Yamasaki and K. Toda, *Knee Surg., Sports Traumatol., Arthrosc.*, 2006, **14**, 1307–1314.
51 K. B. Lee, J. H. Hui, I. C. Song, L. Ardany and E. H. Lee, *Stem Cells*, 2007, **25**, 2964–2971.
52 T. van Haaften, R. Byrne, S. Bonnet, G. Y. Rochefort, J. Akabutu, M. Bouchentouf, G. J. Rey-Parra, J. Galipeau, A. Haromy, F. Eaton, M. Chen, K. Hashimoto, D. Abley, G. Korbutt, S. L. Archer and B. Thebaud, *Am. J. Respir. Crit. Care Med.*, 2009, **180**, 1131–1142.
53 J. L. Liesveld, N. Sharma and O. S. Aljitawi, *Stem Cells*, 2020, **38**, 1241–1253.
54 G. C. Yeo and A. S. Weiss, *Proc. Natl. Acad. Sci. U. S. A.*, 2019, **116**, 2042–2051.
55 M. Ullah, D. D. Liu and A. S. Thakor, *iScience*, 2019, **15**, 421–438.

 This journal is © The Royal Society of Chemistry 2023

Published on 23 may 2023. Downloaded on 28.12.2024 05:50:03.

56 A. De Becker and I. V. Riet, *World J. Stem Cells*, 2016, **8**, 73–87.
57 R. Sackstein, J. S. Merzaban, D. W. Cain, N. M. Dagia, J. A. Spencer, C. P. Lin and R. Wohlgemuth, *Nat. Med.*, 2008, **14**, 181–187.
58 T. T. Lau and D. A. Wang, *Expert Opin. Biol. Ther.*, 2011, **11**, 189–197.
59 F. Belema-Bedada, S. Uchida, A. Martire, S. Kostin and T. Braun, *Cell Stem Cell*, 2008, **2**, 566–575.
60 T. H. Lin, H. H. Liu, T. H. Tsai, C. C. Chen, T. F. Hsieh, S. S. Lee, Y. J. Lee, W. C. Chen and C. H. Tang, *Biochim. Biophys. Acta*, 2013, **1830**, 4917–4927.
61 M. S. Chen, C. Y. Lin, Y. H. Chiu, C. P. Chen, P. J. Tsai and H. S. Wang, *Stem Cells Int.*, 2018, **2018**, 3524759.
62 Y. Wang, W. Fu, S. Zhang, X. He, Z. Liu, D. Gao and T. Xu, *Brain Res.*, 2014, **1575**, 78–86.
63 S. Lourenco, V. H. Teixeira, T. Kalber, R. J. Jose, R. A. Floto and S. M. Janes, *J. Immunol.*, 2015, **194**, 3463–3474.
64 B. R. Son, L. A. Marquez-Curtis, M. Kucia, M. Wysoczynski, A. R. Turner, J. Ratajczak, M. Z. Ratajczak and A. Janowska-Wieczorek, *Stem Cells*, 2006, **24**, 1254–1264.
65 M. Honczarenko, Y. Le, M. Swierkowski, I. Ghiran, A. M. Glodek and L. E. Silberstein, *Stem Cells*, 2006, **24**, 1030–1041.
66 M. Shi, J. Li, L. Liao, B. Chen, B. Li, L. Chen, H. Jia and R. C. Zhao, *Haematologica*, 2007, **92**, 897–904.
67 Y. Li, X. Yu, S. Lin, X. Li, S. Zhang and Y. H. Song, *Biochem. Biophys. Res. Commun.*, 2007, **356**, 780–784.
68 L. K. Tsai, Y. Leng, Z. Wang, P. Leeds and D. M. Chuang, *Neuropsychopharmacology*, 2010, **35**, 2225–2237.
69 L. K. Tsai, Z. Wang, J. Munasinghe, Y. Leng, P. Leeds and D. M. Chuang, *Stroke*, 2011, **42**, 2932–2939.
70 Y. S. Kim, M. Y. Noh, J. Y. Kim, H. J. Yu, K. S. Kim, S. H. Kim and S. H. Koh, *Mol. Neurobiol.*, 2013, **47**, 811–820.
71 H. Fan, G. Zhao, L. Liu, F. Liu, W. Gong, X. Liu, L. Yang, J. Wang and Y. Hou, *Cell. Mol. Immunol.*, 2012, **9**, 473–481.
72 R. Najafi and A. M. Sharifi, *Expert Opin. Biol. Ther.*, 2013, **13**, 959–972.
73 Y. Qiu, L. A. Marquez-Curtis and A. Janowska-Wieczorek, *Cytotherapy*, 2012, **14**, 285–295.
74 M. Duijvestein, M. E. Wildenberg, M. M. Welling, S. Hennink, I. Molendijk, V. L. van Zuylen, T. Bosse, A. C. Vos, E. S. de Jonge-Muller, H. Roelofs, L. van der Weerd, H. W. Verspaget, W. E. Fibbe, A. A. te Velde, G. R. van den Brink and D. W. Hommes, *Stem Cells*, 2011, **29**, 1549–1558.
75 B. Follin, M. Juhl, S. Cohen, A. E. Pedersen, J. Kastrup and A. Ekblond, *Tissue Eng., Part B*, 2016, **22**, 322–329.
76 P. Zhou, Z. Liu, X. Li, B. Zhang, X. Wang, J. Lan, Q. Shi, D. Li and X. Ju, *Biochem. Biophys. Res. Commun.*, 2017, **491**, 323–328.
77 F. Zhao, W. Liu, S. Yue, L. Yang, Q. Hua, Y. Zhou, H. Cheng, Z. Luo and S. Tang, *Stem Cells Int.*, 2019, **2019**, 1726743.
78 L. Ghahari, M. Safari, K. Rahimi Jaberi, B. Jafari, K. Safari and M. Madadian, *Iran. Biomed. J.*, 2020, **24**, 89–98.
79 H. C. Pan, C. J. Chen, F. C. Cheng, S. P. Ho, M. J. Liu, S. M. Hwang, M. H. Chang and Y. C. Wang, *Neurochem. Res.*, 2009, **34**, 518–527.
80 D. Li, Q. Liu, L. Qi, X. Dai, H. Liu and Y. Wang, *Mol. Med. Rep.*, 2016, **14**, 1681–1692.
81 E. J. Lee, H. W. Park, H. J. Jeon, H. S. Kim and M. S. Chang, *Regener. Med.*, 2013, **8**, 283–293.
82 H. Willer, G. Spohn, K. Morgenroth, C. Thielemann, S. Elvers-Hornung, P. Bugert, B. Delorme, M. Giesen, T. Schmitz-Rixen, E. Seifried, C. Pfarrer, R. Schafer and K. Bieback, *Front. Immunol.*, 2022, **13**, 976511.
83 Y. S. Han, J. H. Lee, J. S. Jung, H. Noh, M. J. Baek, J. M. Ryu, Y. M. Yoon, H. J. Han and S. H. Lee, *Int. J. Cardiol.*, 2015, **198**, 187–195.
84 R. Deng, Y. Liu, H. He, H. Zhang, C. Zhao, Z. Cui, Y. Hong, X. Li, F. Lin, D. Yuan, X. Liang and Y. Zhang, *J. Cell. Mol. Med.*, 2020, **24**, 431–440.
85 M. Ejtehadifar, K. Shamsasenjan, A. Movassaghpour, P. Akbarzadehlaleh, N. Dehdilani, P. Abbasi, Z. Molaeipour and M. Saleh, *Adv. Pharm. Bull.*, 2015, **5**, 141–149.
86 N. C. Noronha, A. Mizukami, C. Caliari-Oliveira, J. G. Cominal, J. L. M. Rocha, D. T. Covas, K. Swiech and K. C. R. Malmegrim, *Stem Cell Res. Ther.*, 2019, **10**, 131.
87 B. Annabi, Y. T. Lee, S. Turcotte, E. Naud, R. R. Desrosiers, M. Champagne, N. Eliopoulos, J. Galipeau and R. Beliveau, *Stem Cells*, 2003, **21**, 337–347.
88 H. Liu, W. Xue, G. Ge, X. Luo, Y. Li, H. Xiang, X. Ding, P. Tian and X. Tian, *Biochem. Biophys. Res. Commun.*, 2010, **401**, 509–515.
89 S. C. Hung, R. R. Pochampally, S. C. Hsu, C. Sanchez, S. C. Chen, J. Spees and D. J. Prockop, *PLoS One*, 2007, **2**, e416.
90 S. S. Meng, X. P. Xu, W. Chang, Z. H. Lu, L. L. Huang, J. Y. Xu, L. Liu, H. B. Qiu, Y. Yang and F. M. Guo, *Stem Cell Res. Ther.*, 2018, **9**, 280.
91 G. Vertelov, L. Kharazi, M. G. Muralidhar, G. Sanati, T. Tankovich and A. Kharazi, *Stem Cell Res. Ther.*, 2013, **4**, 5.
92 L. F. Raheja, D. C. Genetos, A. Wong and C. E. Yellowley, *Cell Biol. Int.*, 2011, **35**, 981–989.
93 J. Beegle, K. Lakatos, S. Kalomoiris, H. Stewart, R. R. Isseroff, J. A. Nolta and F. A. Fierro, *Stem Cells*, 2015, **33**, 1818–1828.
94 Y. W. Lan, K. B. Choo, C. M. Chen, T. H. Hung, Y. B. Chen, C. H. Hsieh, H. P. Kuo and K. Y. Chong, *Stem Cell Res. Ther.*, 2015, **6**, 97.
95 J. Sun, Z. Z. Wei, X. Gu, J. Y. Zhang, Y. Zhang, J. Li and L. Wei, *Exp. Neurol.*, 2015, **272**, 78–87.
96 I. Moniz, J. Ramalho-Santos and A. F. Branco, *Int. J. Mol. Sci.*, 2022, **23**, 3749.
97 C. W. Lee, D. Kang, A. K. Kim, D. Y. Kim and D. I. Kim, *Int. J. Stem Cells*, 2018, **11**, 61–67.
98 S. E. Park, H. Kim, S. Kwon, S. J. Choi, S. Y. Oh, G. H. Ryu, H. B. Jeon and J. W. Chang, *Int. J. Mol. Sci.*, 2020, **21**, 7092.

Published on 23 may 2023. Downloaded on 28.12.2024 05:50:03.

99 H. Obradovic, J. Krstic, D. Trivanovic, S. Mojsilovic, I. Okic, T. Kukolj, V. Ilic, A. Jaukovic, M. Terzic and D. Bugarski, *Placenta*, 2019, **82**, 25–34.
100 A. Matta, V. Nader, M. Lebrin, F. Gross, A. C. Prats, D. Cussac, M. Galinier and J. Roncalli, *Cells*, 2022, **11**, 1620.
101 R. R. Pochampally, J. R. Smith, J. Ylostalo and D. J. Prockop, *Blood*, 2004, **103**, 1647–1652.
102 A. Oskowitz, H. McFerrin, M. Gutschow, M. L. Carter and R. Pochampally, *Stem Cell Res.*, 2011, **6**, 215–225.
103 S. Takao, T. Nakashima, T. Masuda, M. Namba, S. Sakamoto, K. Yamaguchi, Y. Horimasu, S. Miyamoto, H. Iwamoto, K. Fujitaka, H. Hamada, S. Takahashi, A. Nakashima and N. Hattori, *Stem Cell Res. Ther.*, 2021, **12**, 506.
104 X. Wu, D. Wu, Y. Mu, Y. Zhao and Z. Ma, *Front. Bioeng. Biotechnol.*, 2020, **8**, 586.
105 K. M. Holmstrom and T. Finkel, *Nat. Rev. Mol. Cell Biol.*, 2014, **15**, 411–421.
106 S. G. Rhee, *Science*, 2006, **312**, 1882–1883.
107 F. Nouri, S. N. Nematollahi-Mahani and A. M. Shari fi, *Adv. Pharm. Bull.*, 2019, **9**, 76–83.
108 L. Guo, J. Du, D. F. Yuan, Y. Zhang, S. Zhang, H. C. Zhang, J. W. Mi, Y. L. Ning, M. J. Chen, D. L. Wen, J. H. Sun, D. Liu, L. Zeng, A. Zhang, J. Jiang and H. Huang, *Stem Cell Res. Ther.*, 2020, **11**, 434.
109 T. Mahmoudi, K. Abdolmohammadi, H. Bashiri, M. Mohammadi, M. J. Rezaie, F. Fathi, S. Fakhari, M. A. Rezaee, A. Jalili, M. R. Rahmani and L. Tayebi, *Adv. Pharm. Bull.*, 2020, **10**, 72–80.
110 Y. W. Won, A. N. Patel and D. A. Bull, *Biomaterials*, 2014, **35**, 5627–5635.
111 W. J. Rombouts and R. E. Ploemacher, *Leukemia*, 2003, **17**, 160–170.
112 D. Sarkar, W. Zhao, A. Gupta, W. L. Loh, R. Karnik and J. M. Karp, *Methods Mol. Biol.*, 2011, **698**, 505–523.
113 I. K. Ko, B. G. Kim, A. Awadallah, J. Mikulan, P. Lin, J. J. Letterio and J. E. Dennis, *Mol. Ther.*, 2010, **18**, 1365–1372.
114 N. Nayerossadat, T. Maedeh and P. A. Ali, *Adv. Biomed. Res.*, 2012, **1**, 27.
115 D. Y. Lee, B. H. Cha, M. Jung, A. S. Kim, D. A. Bull and Y. W. Won, *J. Biol. Eng.*, 2018, **12**, 28.
116 T. M. Lanigan, H. C. Kopera and T. L. Saunders, *Genes*, 2020, **11**, 291.
117 Z. Cheng, L. Ou, X. Zhou, F. Li, X. Jia, Y. Zhang, X. Liu, Y. Li, C. A. Ward, L. G. Melo and D. Kong, *Mol. Ther.*, 2008, **16**, 571–579.
118 Q. Jiang, K. Huang, F. Lu, S. Deng, Z. Yang and S. Hu, *Gen. Thorac. Cardiovasc. Surg.*, 2022, **70**, 1–10.
119 J. X. Yang, N. Zhang, H. W. Wang, P. Gao, Q. P. Yang and Q. P. Wen, *J. Biol. Chem.*, 2015, **290**, 1994–2006.
120 X. Huang, F. Zhang, Y. Wang, X. Sun, K. Y. Choi, D. Liu, J. S. Choi, T. H. Shin, J. Cheon, G. Niu and X. Chen, *ACS Nano*, 2014, **8**, 4403–4414.
121 T. Zhang, F. Li, Q. Xu, Q. Wang, X. Jiang, Z. Liang, H. Liao, X. Kong, J. Liu, H. Wu, D. Zhang, C. An, L. Dong, Y. Lu, H. Cao, D. Kim, J. Sun, T. Hyeon, J. Gao and D. Ling, *Adv. Funct. Mater.*, 2019, **29**, 1900603.
122 J. Huang, Z. Zhang, J. Guo, A. Ni, A. Deb, L. Zhang, M. Mirotsou, R. E. Pratt and V. J. Dzau, *Circ. Res.*, 2010, **106**, 1753–1762.
123 Y. Gao, A. Yao, W. Zhang, S. Lu, Y. Yu, L. Deng, A. Yin, Y. Xia, B. Sun and X. Wang, *Oncogene*, 2010, **29**, 2784–2794.
124 H. Chen, S. Tang, J. Liao, M. Liu and Y. Lin, *J. Gene Med.*, 2021, **23**, e3369.
125 Y. Zhao, J. Zhu, N. Zhang, Q. Liu, Y. Wang, X. Hu, J. Chen, W. Zhu and H. Yu, *Stem Cells Transl. Med.*, 2020, **9**, 1257–1271.
126 H. Song, K. Kwon, S. Lim, S. M. Kang, Y. G. Ko, Z. Xu, J. H. Chung, B. S. Kim, H. Lee, B. Joung, S. Park, D. Choi, Y. Jang, N. S. Chung, K. J. Yoo and K. C. Hwang, *Mol. Cells*, 2005, **19**, 402–407.
127 B. M. Marte and J. Downward, *Trends Biochem. Sci.*, 1997, **22**, 355–358.
128 L. Zhou, S. Liu, Z. Wang, J. Yao, W. Cao, S. Chen, W. Xie, S. Feng, Y. Xu, T. Cheng, M. Han and S. Feng, *Biol. Proced. Online*, 2019, **21**, 24.
129 A. A. Mangi, N. Noiseux, D. Kong, H. He, M. Rezvani, J. S. Ingwall and V. J. Dzau, *Nat. Med.*, 2003, **9**, 1195–1201.
130 W. Li, N. Ma, L. L. Ong, C. Nesselmann, C. Klopsch, Y. Ladilov, D. Furlani, C. Piechaczek, J. M. Moebius, K. Lutzow, A. Lendlein, C. Stamm, R. K. Li and G. Steinhoff, *Stem Cells*, 2007, **25**, 2118–2127.
131 X. Ni, C. Ou, J. Guo, B. Liu, J. Zhang, Z. Wu, H. Li and M. Chen, *Int. J. Mol. Med.*, 2017, **40**, 418–426.
132 H. He, Z. H. Zhao, F. S. Han, X. H. Liu, R. Wang and Y. J. Zeng, *Cell Death Dis.*, 2016, **7**, e2056.
133 L. McGinley, J. McMahon, P. Strappe, F. Barry, M. Murphy, D. O'Toole and T. O'Brien, *Stem Cell Res. Ther.*, 2011, **2**, 12.
134 X. Wang, T. Zhao, W. Huang, T. Wang, J. Qian, M. Xu, E. G. Kranias, Y. Wang and G. C. Fan, *Stem Cells*, 2009, **27**, 3021–3031.
135 Y. L. Tang, Y. Tang, Y. C. Zhang, K. Qian, L. Shen and M. I. Phillips, *J. Am. Coll. Cardiol.*, 2005, **46**, 1339–1350.
136 N. Liu, H. Wang, G. Han, J. Cheng, W. Hu and J. Zhang, *Int. J. Mol. Med.*, 2018, **42**, 946–956.
137 Y. Xing, J. Hou, T. Guo, S. Zheng, C. Zhou, H. Huang, Y. Chen, K. Sun, T. Zhong, J. Wang, H. Li and T. Wang, *Stem Cell Res. Ther.*, 2014, **5**, 130.
138 Y. Zhao, X. Yang, S. Li, B. Zhang, S. Li, X. Wang, Y. Wang, C. Jia, Y. Chang and W. Wei, *Stem Cell Res. Ther.*, 2021, **12**, 1–23.
139 O. Levy, W. Zhao, L. J. Mortensen, S. Leblanc, K. Tsang, M. Fu, J. A. Phillips, V. Sagar, P. Anandakumaran, J. Ngai, C. H. Cui, P. Eimon, M. Angel, C. P. Lin, M. F. Yanik and J. M. Karp, *Blood*, 2013, **122**, e23–e32.
140 W. Liao, V. Pham, L. Liu, M. Riazifar, E. J. Pone, S. X. Zhang, F. Ma, M. Lu, C. M. Walsh and W. Zhao, *Biomaterials*, 2016, **77**, 87–97.
141 J. S. Park, S. Suryaprakash, Y. H. Lao and K. W. Leong, *Methods*, 2015, **84**, 3–16.

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142 J. M. McKinney, K. A. Pucha, T. N. Doan, L. Wang, L. D. Weinstock, B. T. Tignor, K. L. Fowle, R. D. Levit, L. B. Wood and N. J. Willett, *Acta Biomater.*, 2022, **141**, 315–332.
143 X. Ke, M. Li, X. Wang, J. Liang, X. Wang, S. Wu, M. Long and C. Hu, *Carbohydr. Polym.*, 2020, **229**, 115516.
144 B. Wang, W. Liu, J. J. Li, S. Chai, D. Xing, H. Yu, Y. Zhang, W. Yan, Z. Xu, B. Zhao, Y. Du and Q. Jiang, *Bioact. Mater.*, 2022, **7**, 478–490.
145 Y. Lyu, J. Xie, Y. Liu, M. Xiao, Y. Li, J. Yang, J. Yang and W. Liu, *ACS Biomater. Sci. Eng.*, 2020, **6**, 6926–6937.
146 Y. Jiao, X. Chen, B. Nong, M. Luo, Y. Niu, S. Huang, J. Zhang, A. Wei and J. Huang, *J. Mater. Chem. B*, 2022, **10**, 8330–8346.
147 Q. Xu, A. Sigen, Y. Gao, L. Guo, J. Creagh-Flynn, D. Zhou, U. Greiser, Y. Dong, F. Wang, H. Tai, W. Liu, W. Wang and W. Wang, *Acta Biomater.*, 2018, **75**, 63–74.
148 K. Lau, A. Waterhouse, B. Akhavan, L. Gao, H. N. Kim, F. Tang, J. M. Whitelock, M. M. Bilek, M. S. Lord and J. Rnjak-Kovacina, *Acta Biomater.*, 2021, **132**, 162–175.
149 G. Janani, M. Kumar, D. Chouhan, J. C. Moses, A. Gangrade, S. Bhattacharjee and B. B. Mandal, *ACS Appl. Bio Mater.*, 2019, **2**, 5460–5491.
150 C. Holland, K. Numata, J. Rnjak-Kovacina and F. P. Seib, *Adv. Healthcare Mater.*, 2019, **8**, e1800465.
151 L. Wang, Y. Liu, G. Ye, Y. He, B. Li, Y. Guan, B. Gong, K. Mequanint, M. M. Q. Xing and X. Qiu, *Nat. Biomed. Eng.*, 2021, **5**, 1157–1173.
152 H. Almeida, R. M. A. Domingues, S. M. Mithieux, R. A. Pires, A. I. Goncalves, M. Gomez-Florit, R. L. Reis, A. S. Weiss and M. E. Gomes, *ACS Appl. Mater. Interfaces*, 2019, **11**, 19830–19840.
153 G. C. Yeo, E. Kosobrodova, A. Kondyurin, D. R. McKenzie, M. M. Bilek and A. S. Weiss, *Macromol. Biosci.*, 2019, **19**, e1800233.
154 K. Joyce, G. T. Fabra, Y. Bozkurt and A. Pandit, *Signal Transduction Targeted Ther.*, 2021, **6**, 122.
155 J. R. Martin, P. Patil, F. Yu, M. K. Gupta and C. L. Duvall, *Biomaterials*, 2020, **263**, 120377.
156 X. Mei, D. Zhu, J. Li, K. Huang, S. Hu, Z. Li, B. Lopez de Juan Abad and K. Cheng, *Med*, 2021, **2**, 1253–1268.
157 M. Camara-Torres, R. Sinha, C. Mota and L. Moroni, *Acta Biomater.*, 2020, **101**, 183–195.
158 K. U. Wong, A. Zhang, B. Akhavan, M. M. Bilek and G. C. Yeo, *ACS Biomater. Sci. Eng.*, 2021, DOI: **10.1021/acsbiomaterials.0c01538**.
159 M. R. Casanova, R. L. Reis, A. Martins and N. M. Neves, *Mater. Horiz.*, 2020, **7**, 2258–2275.
160 B. N. Kharbikar, P. Mohindra and T. A. Desai, *Cell Stem Cell*, 2022, **29**, 692–721.
161 US Food and Drug Administration, Biological evaluation of medical devices - Part 1: Evaluation and testing within a risk management process, 2020, 68.
162 E. Marin, F. Boschetto and G. Pezzotti, *J. Biomed. Mater. Res., Part A*, 2020, **108**, 1617–1633.
163 M. Bai, L. Cai, X. Li, L. Ye and J. Xie, *J. Biomed. Mater. Res., Part B*, 2020, **108**, 2426–2440.
164 K. H. Vining and D. J. Mooney, *Nat. Rev. Mol. Cell Biol.*, 2017, **18**, 728–742.
165 T. P. Liu, P. Ha, C. Y. Xiao, S. Y. Kim, A. R. Jensen, J. Easley, Q. Yao and X. Zhang, *Front. Cell Dev. Biol.*, 2022, **10**, 982199.
166 L. He and X. Chen, *Adv. Healthcare Mater.*, 2020, **9**, e2001175.
167 K. Niveditha, C. A. Vineeth, J. Joseph, A. Unnikrishna Pillai, A. John and A. Abraham, *Curr. Stem Cell Res. Ther.*, 2021, **16**, 155–164.
168 S. V. Murphy, P. De Coppi and A. Atala, *Nat. Biomed. Eng.*, 2020, **4**, 370–380.
169 M. C. Ciuffreda, G. Malpasso, C. Chokoza, D. Bezuidenhout, K. P. Goetsch, M. Mura, F. Pisano, N. H. Davies and M. Gnecchi, *Acta Biomater.*, 2018, **70**, 71–83.
170 W. Hu, Z. Wang, Y. Xiao, S. Zhang and J. Wang, *Biomater. Sci.*, 2019, **7**, 843–855.
171 J. Y. C. Lim, Q. Lin, K. Xue and X. J. Loh, *Mater. Today Adv.*, 2019, **3**, 100021.
172 R. Levato and K. S. Lim, *Biofabrication*, 2023, **15**, 020401.
173 H. Lee, T. W. Lee, A. Chandrasekharan, S. E. Sung, S. G. Yim, S. Kim, K. Y. Seong, M. S. Seo and S. Y. Yang, *ACS Biomater. Sci. Eng.*, 2022, **8**, 1613–1622.
174 L. Figueiredo, R. Pace, C. D'Arros, G. Rethore, J. Guicheux, C. Le Visage and P. Weiss, *J. Tissue Eng. Regener. Med.*, 2018, **12**, 1238–1246.
175 J. Li and D. J. Mooney, *Nat. Rev. Mater.*, 2016, **1**, 16071.
176 D. N. Heo, M. Hospodiuk and I. T. Ozbolat, *Acta Biomater.*, 2019, **95**, 348–356.
177 V. G. Muir, S. Weintraub, A. P. Dhand, H. Fallahi, L. Han and J. A. Burdick, *Adv. Sci.*, 2023, **10**, e2206117.
178 V. G. Muir, T. H. Qazi, S. Weintraub, B. O. Torres Maldonado, P. E. Arratia and J. A. Burdick, *Small*, 2022, **18**, e2201115.
179 J. Koh, D. R. Griffin, M. M. Archang, A. C. Feng, T. Horn, M. Margolis, D. Zalazar, T. Segura, P. O. Scumpia and D. Di Carlo, *Small*, 2019, **15**, e1903147.
180 D. W. Kim, H. S. Jeong, E. Kim, H. Lee, C. H. Choi and S. J. Lee, *J. Controlled Release*, 2022, **347**, 508–520.
181 S. Akbari, T. Pirbodaghi, R. D. Kamm and P. T. Hammond, *Lab Chip*, 2017, **17**, 2067–2075.
182 D. M. Headen, J. R. García and A. J. García, *Microsyst. Nanoeng.*, 2018, **4**, 17076.
183 C. X. Luan, P. Liu, R. Z. Chen and B. A. Chen, *Nanotechnol. Rev.*, 2017, **6**, 435–448.
184 J. Li, S. Hu, D. Zhu, K. Huang, X. Mei, B. Lopez de Juan Abad and K. Cheng, *J. Am. Heart Assoc.*, 2021, **10**, e020402.
185 B. Wahlberg, H. Ghuman, J. R. Liu and M. Modo, *Sci. Rep.*, 2018, **8**, 9194.
186 M. H. Amer, L. J. White and K. M. Shakesheff, *J. Pharm. Pharmacol.*, 2015, **67**, 640–650.
187 B. A. Aguado, W. Mulyasasmita, J. Su, K. J. Lampe and S. C. Heilshorn, *Tissue Eng., Part A*, 2012, **18**, 806–815.

Published on 23 may 2023. Downloaded on 28.12.2024 05:50:03.

188 S. Soltani, R. Emadi, S. Haghjooy Javanmard, M. Kharaziha, A. Rahmati, V. K. Thakur and S. Lotfian, *Gels*, 2022, **8**, 121.
189 S. Zhang, D. Huang, H. Lin, Y. Xiao and X. Zhang, *Biomacromolecules*, 2020, **21**, 2400–2408.
190 L. Cai, R. E. Dewi and S. C. Heilshorn, *Adv. Funct. Mater.*, 2015, **25**, 1344–1351.
191 K. Adu-Berchie and D. J. Mooney, *Acc. Chem. Res.*, 2020, **53**, 1749–1760.
192 S. E. D'Souza, M. H. Ginsberg and E. F. Plow, *Trends Biochem. Sci.*, 1991, **16**, 246–250.
193 S. Sahab Negah, A. Khooei, F. Samini and A. Gorji, *Cell Tissue Res.*, 2018, **371**, 223–236.
194 C. Addi, F. Murschel and G. De Crescenzo, *Tissue Eng., Part B*, 2017, **23**, 163–182.
195 A. J. Matsuoka, Z. A. Sayed, N. Stephanopoulos, E. J. Berns, A. R. Wadhwani, Z. D. Morrissey, D. M. Chadly, S. Kobayashi, A. N. Edelbrock, T. Mashimo, C. A. Miller, T. L. McGuire, S. I. Stupp and J. A. Kessler, *PLoS One*, 2017, **12**, e0190150.
196 X. Cao, L. Duan, H. Hou, Y. Liu, S. Chen, S. Zhang, Y. Liu, C. Wang, X. Qi, N. Liu, Z. Han, D. Zhang, Z. C. Han, Z. Guo, Q. Zhao and Z. Li, *Theranostics*, 2020, **10**, 7697–7709.
197 G. Li, Z. M. Li, L. L. Li, S. Liu, P. Wu, M. Zhou, C. W. Li, X. D. Li, G. X. Luo and J. X. Zhang, *Adv. Funct. Mater.*, 2023, **33**, 2209466.
198 Y. Zhao, C. Gao, H. Liu, H. Liu, Y. Feng, Z. Li, H. Liu, J. Wang, B. Yang and Q. Lin, *Acta Biomater.*, 2021, **121**, 653–664.
199 T. Thum, J. Bauersachs, P. A. Poole-Wilson, H. D. Volk and S. D. Anker, *J. Am. Coll. Cardiol.*, 2005, **46**, 1799–1802.
200 M. B. Preda, C. A. Neculachi, I. M. Fenyo, A. M. Vacaru, M. A. Publik, M. Simionescu and A. Burlacu, *Cell Death Dis.*, 2021, **12**, 566.
201 S. Daly, M. Thorpe, S. Rockswold, M. Hubbard, T. Bergman, U. Samadani and G. Rockswold, *J. Neurotrauma*, 2018, **35**, 623–629.
202 M. M. Coronel, R. Geusz and C. L. Stabler, *Biomaterials*, 2017, **129**, 139–151.
203 S. H. Oh, C. L. Ward, A. Atala, J. J. Yoo and B. S. Harrison, *Biomaterials*, 2009, **30**, 757–762.
204 B. S. Harrison, D. Eberli, S. J. Lee, A. Atala and J. J. Yoo, *Biomaterials*, 2007, **28**, 4628–4634.
205 S. M. Ng, J. Y. Choi, H. S. Han, J. S. Huh and J. O. Lim, *Int. J. Pharm.*, 2010, **384**, 120–127.
206 P. E. Keipert, *Adv. Exp. Med. Biol.*, 2017, **977**, 343–350.
207 Y. Wang, E. R. Zoneff, J. W. Thomas, N. Hong, L. L. Tan, D. J. McGillivray, A. W. Perriman, K. C. L. Law, L. H. Thompson, N. Moriarty, C. L. Parish, R. J. Williams, C. J. Jackson and D. R. Nisbet, *Nat. Commun.*, 2023, **14**, 457.
208 H. Niu, C. Li, Y. Guan, Y. Dang, X. Li, Z. Fan, J. Shen, L. Ma and J. Guan, *Acta Biomater.*, 2020, **105**, 56–67.
209 A. Moya, J. Paquet, M. Deschepper, N. Larochette, K. Oudina, C. Denoeud, M. Bensidhoum, D. Logeart-Avramoglou and H. Petite, *Stem Cells*, 2018, **36**, 363–376.
210 P. Galvez, M. J. Martin, A. C. Calpena, J. A. Tamayo, M. A. Ruiz and B. Clares, *Pharm. Res.*, 2014, **31**, 3515–3528.
211 M. Zargarzadeh, A. S. Silva, C. Nunes, M. A. Coimbra, C. A. Custodio and J. F. Mano, *Mater. Horiz.*, 2022, **9**, 694–707.
212 S. Zhu, C. Yu, N. Liu, M. Zhao, Z. Chen, J. Liu, G. Li, H. Huang, H. Guo, T. Sun, J. Chen, J. Zhuang and P. Zhu, *Bioact. Mater.*, 2022, **13**, 119–134.
213 A. Huang, D. Liu, X. Qi, Z. Yue, H. Cao, K. Zhang, X. Lei, Y. Wang, D. Kong, J. Gao, Z. Li, N. Liu and Y. Wang, *Acta Biomater.*, 2019, **85**, 94–105.
214 B. Xu, Y. Cao, Z. Zheng, E. A. Galan, Z. Hu, J. Ge, X. Xing and S. Ma, *Adv. Biol.*, 2021, **5**, e2000202.
215 J. Wang, D. Huang, H. Ren and L. Shang, *Small*, 2022, **18**, e2200858.
216 J. Park, G. Choe, S. Oh and J. Y. Lee, *ACS Biomater. Sci. Eng.*, 2020, **6**, 6938–6948.
217 K. Luo, X. Gao, Y. Gao, Y. Li, M. Deng, J. Tan, J. Gou, C. Liu, C. Dou, Z. Li, Z. Zhang, J. Xu and F. Luo, *Acta Biomater.*, 2019, **85**, 106–116.
218 I. E. Epanomeritakis, E. Lee, V. Lu and W. Khan, *Int. J. Mol. Sci.*, 2022, **23**, 4065.
219 M. Lian, B. Sun, Y. Han, B. Yu, W. Xin, R. Xu, B. Ni, W. Jiang, Y. Hao, X. Zhang, Y. Shen, Z. Qiao and K. Dai, *Biomaterials*, 2021, **274**, 120841.
220 D. Garcia-Sanchez, D. Fernandez, J. C. Rodriguez-Rey and F. M. Perez-Campo, *World J. Stem Cells*, 2019, **11**, 748–763.
221 S. Du, D. I. Zeugolis and T. O'Brien, *Stem Cell Res. Ther.*, 2022, **13**, 426.
222 Y. Tang, H. Wang, Y. Sun, Y. Jiang, S. Fang, Z. Kan, Y. Lu, S. Liu, X. Zhou and Z. Li, *ACS Appl. Bio Mater.*, 2021, **4**, 8607–8614.
223 S. L. Hirsh, D. R. McKenzie, N. J. Nosworthy, J. A. Denman, O. U. Sezerman and M. M. Bilek, *Colloids Surf., B*, 2013, **103**, 395–404.
224 M. J. Ainsworth, O. Lotz, A. Gilmour, A. Zhang, M. J. Chen, D. R. McKenzie, M. M. M. Bilek, J. Malda, B. Akhavan and M. Castilho, *Adv. Funct. Mater.*, 2022, **33**, 2206583.
225 K. Redzikultsava, M. Baldry, A. Y. Zhang, S. K. H. Alavi, B. Akhavan and M. M. Bilek, *Plasma Processes Polym.*, 2022, **19**, e2200018.
226 E. Kosobrodova, A. Kondyurin, V. Solodko, A. S. Weiss, D. R. McKenzie and M. M. M. Bilek, *ACS Appl. Mater. Interfaces*, 2020, **12**, 32163–32174.
227 Z. Wang, L. Liu, S. M. Mithieux and A. S. Weiss, *Trends Biotechnol.*, 2021, **39**, 505–518.
228 Z. Wang, S. M. Mithieux, H. Vindin, Y. Wang, M. Zhang, L. Liu, J. Zbinden, K. M. Blum, T. Yi, Y. Matsuzaki, F. Oveissi, R. Akdemir, K. M. Lockley, L. Zhang, K. Ma, J. Guan, A. Waterhouse, N. T. H. Pham, B. S. Hawkett, T. Shinoka, C. K. Breuer and A. S. Weiss, *Adv. Mater.*, 2022, **34**, e2205614.
229 L. N. Zamproni, M. Grinet, M. Mundim, M. B. C. Reis, L. T. Galindo, F. R. Marciano, A. O. Lobo and M. Porcionatto, *Nanomedicine*, 2019, **15**, 98–107.

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230 Y. C. Chiang, H. W. Yeh, S. M. Hu, C. Y. Wu, T. Y. Wu, C. H. Chen, P. C. Liao, Z. Y. Guan, N. C. Cheng and H. Y. Chen, *Mater. Today Bio*, 2022, **13**, 100213.
231 Y. Wang, C. Xie, Z. Zhang, H. Liu, H. Xu, Z. Peng, C. Liu, J. Li, C. Wang, T. Xu and L. Zhu, *ACS Appl. Mater. Interfaces*, 2022, **14**, 29506–29520.
232 G. Boix-Lemonche, R. M. Nagymihaly, E. M. Niemi, N. Josifovska, S. Johansen, M. C. Moe, H. Scholz and G. Petrovski, *Macromol. Biosci.*, 2023, e2200422, DOI: 10.1002/mabi.202200422.
233 J. Huang, Z. Huang, Y. Liang, W. Yuan, L. Bian, L. Duan, Z. Rong, J. Xiong, D. Wang and J. Xia, *Biomater. Sci.*, 2021, **9**, 2620–2630.
234 R. Bushkalova, M. Farno, C. Tenailleau, B. Duployer, D. Cussac, A. Parini, B. Sallerin and S. Girod Fullana, *Int. J. Pharm.*, 2019, **571**, 118692.
235 A. Czosseck, M. M. Chen, H. Nguyen, A. Meeson, C. C. Hsu, C. C. Chen, T. A. George, S. C. Ruan, Y. Y. Cheng, P. J. Lin, P. C. H. Hsieh and D. J. Lundy, *J. Controlled Release*, 2022, **352**, 879–892.
236 W. Whyte, E. T. Roche, C. E. Varela, K. Mendez, S. Islam, H. O'Neill, F. Weafer, R. N. Shirazi, J. C. Weaver, N. V. Vasilyev, P. E. McHugh, B. Murphy, G. P. Duffy, C. J. Walsh and D. J. Mooney, *Nat. Biomed. Eng.*, 2018, **2**, 416–428.
237 C. Stewart, B. Akhavan, S. G. Wise and M. M. M. Bilek, *Prog. Mater. Sci.*, 2019, **106**, 100588.
238 R. Dwivedi, S. Kumar, R. Pandey, A. Mahajan, D. Nandana, D. S. Katti and D. Mehrotra, *J. Oral Biol. Craniofac. Res.*, 2020, **10**, 381–388.
239 S. W. Lee, K. S. Phillips, H. Gu, M. Kazemzadeh-Narbat and D. Ren, *Biomaterials*, 2021, **268**, 120595.
240 J. O. Abaricia, N. Farzad, T. J. Heath, J. Simmons, L. Morandini and R. Olivares-Navarrete, *Acta Biomater.*, 2021, **133**, 58–73.
241 X. Dong, S. Liu, Y. Yang, S. Gao, W. Li, J. Cao, Y. Wan, Z. Huang, G. Fan, Q. Chen, H. Wang, M. Zhu and D. Kong, *Biomaterials*, 2021, **272**, 120767.

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