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Engineering Mesenchymal Stem Cells for Regenerative Medicine and Drug Delivery

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Abstract

Researchers have applied mesenchymal stem cells (MSC) to a variety of therapeutic scenarios by harnessing their multipotent, regenerative, and immunosuppressive properties with tropisms toward inflamed, hypoxic, and cancerous sites. Although MSC-based therapies have been shown to be safe and effective to a certain degree, the efficacy remains low in most cases when MSC are applied alone. To enhance their therapeutic efficacy, researchers have equipped MSC with targeted delivery functions using genetic engineering, therapeutic agent incorporation, and cell surface modification. MSC can be genetically modified virally or non-virally to overexpress therapeutic proteins that complement their innate properties. MSC can also be primed with non-peptidic drugs or magnetic nanoparticles for enhanced efficacy and externally regulated targeting, respectively. Furthermore, MSC can be functionalized with targeting moieties to augment their homing toward therapeutic sites using enzymatic modification, chemical conjugation, or non-covalent interactions. These engineering techniques are still works in progress, requiring optimization to improve the therapeutic efficacy and targeting effectiveness while minimizing any loss of MSC function. In this review, we will highlight the advanced techniques of engineering MSC, describe their promise and the challenges of translation into clinical settings, and suggest future perspectives on realizing their full potential for MSC-based therapy.

Keywords

Mesenchymal stem cells (MSC); Cell-based therapy; Tissue engineering; Genetic modification; Nanoparticle (NP); Surface modification

1. Introduction

Mesenchymal stem cells (MSC) are adult stem cells capable of self-renewal and differentiation into multiple lineages including cartilage, adipose, and bone. MSC are

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characterized by their ability to adhere to plastics under standard cell culture conditions, expressing CD44, CD73, CD90, CD105, but not CD45, CD34, and CD14 [1].

Fridenstein first reported MSC merely as proliferating fibroblastic cells from bone marrow capable of differentiating into osteoblasts, chondrocytes and adipocytes. Along with their self-renewal property, MSC secrete factors, such as growth factors, both in an autocrine and paracrine fashion, which affect the surrounding microenvironment to promote angiogenesis, decrease inflammation, and enhance tissue repair. Moreover, MSC exert strong immunosuppressive properties, allowing them to be transplanted without any pre- or post-treatment. Additionally, they are easy to expand in culture and have multi-lineage differentiation potential and tropism toward neo-angiogenic, tumor, and inflammatory sites. MSC also pose no risk of teratoma formation, nor are there any ethical issues associated with the cell source. All of these properties collectively make MSC an attractive candidate for cell-based therapies.

MSC have been isolated from a wide range of sources including bone marrow (BM) [2], umbilical cord (UC), adipose tissue [3], liver [4], multiple dental tissues [5], and induced pluripotent stem cells (iPSC) [6]. Each of these sources has its own advantages and disadvantages. BM is the most characterized and documented source of MSC. However, the collection of MSC from BM is painful, invasive, and characterized by a low yield [2]. MSC in the UC can be obtained from Wharton jelly, veins, arteries, the UC lining, and the subamnion and perivascular regions. UC-derived MSC (UC-MSC) can be obtained through a painless collection method and have fewer associated ethical issues. They also renew faster than BM-derived MSC (BM-MSC) [7]. Adipose tissue is another popular source, mainly because a large number of MSC can be obtained through minimally invasive methods [3]. In all cases, these cells need to be monitored regularly to ascertain their quality. While there are many sources for MSC, the quality of the MSC is highly variable from donor to donor and is significantly affected by age and aging disorders. Furthermore, extended handling of MSC *in vitro* reduces their differentiation potential. To circumvent these issues, MSC were recently derived from iPSC [6]. These cells have the same *in vitro* and *in vivo* characteristics of BM-MSC, such as the potential for adipogenesis, osteogenesis, and chondrogenesis. MSC derived from iPSC also display higher capacity for proliferation and stronger telomerase activity, leading to better engraftment and survival after transplantation. Additionally, they display superior capabilities in repairing tissue ischemia compared to BM-MSC [6]. In addition to tissue regeneration, MSC have been used to treat type-1 diabetes [8], myocardial infarction [9], graft-versus-host disease [10], inflammatory bowel disease [11], and cancer [12]. Currently, there are 395 ongoing or completed clinical trials worldwide using MSC or mesenchymal stromal cells [13], indicating the popularity of MSC for cell-based therapies.

In this review, we will highlight the advanced techniques used to engineer MSC for tissue engineering and drug delivery applications. The challenges and advantages of each technique will also be analyzed and discussed. Numerous clinical trials have established the safety of using MSC for cell-based therapies. However, the efficacy of MSC *in vivo* is still low due to poor survival, retention, and engraftment of the cells. The first section of this paper focuses on genetic modification to enhance the survival, migration, and secretion of

growth factors for their application in the field of regenerative medicine. This is followed by a discussion of MSC applications in cancer therapy and gene therapy. Although genetic modification is a powerful tool, only protein-based drugs can be delivered using this approach. Additionally, the genetic modification could potentially affect the innate properties of MSC. Hence, over the last few years, nanoparticle (NP)-based MSC delivery systems have gained increasing attention. While numerous synthetic NP platforms have been designed and some have even shown promising clinical outcomes, obstacles (including toxicity, specificity, and delivery efficiency) remain to be overcome before translation. In contrast, MSC offer intrinsic homing properties, low toxicity, and low immunogenicity, which could lead to higher delivery efficiency compared to conventional nanomedicine platforms. The second section of the paper focuses on combining conventional NP platforms with MSC-based therapies. The various methods used to load the therapeutic agents onto MSC, release the therapeutic agents from MSC, and the applications of such MSC-NP combination are analyzed in detail. However, NP-based MSC therapy must ensure that the NP does not compromise the cell's native properties and it can deliver a suitable release profile. To deal with these issues, researchers have used surface modification of MSC as an alternative. Using various engineering approaches (enzymatic modification, chemical modification, and non-covalent interactions), researchers immobilize targeting moieties onto the cell surface to direct MSC to the therapeutic site. As surface modification confers only transient expression of targeting molecules on MSC, it does not significantly affect the cells' phenotype. The last section will suggest future perspectives for translating MSC-based therapies.

2. Techniques for Engineering MSC

2.1. Genetic Modifications

The clinical application of MSC is often hampered by inadequate *in vivo* performance with respect to survival, retention, and engraftment. Genetic engineering is one approach to improve the *in vivo* performance of MSC. MSC are genetically engineered to secrete factors that can protect MSC from apoptosis, increase their survivability in hypoxic conditions, and enhance other innate properties, such as migration, cardiac protection, and differentiation to a particular lineage. Moreover, genetic modifications have also been used to engineer MSC to produce therapeutic proteins for treating diseases like hemophilia and diabetes, and for repairing musculoskeletal disorders. Genetic modification of MSC is usually achieved *via* viral vectors although the use of non-viral vectors is on the rise.

2.1.1. Viral transduction—MSC are readily amenable to viral modification. Standard protocols can lead to 90 % transduced cells with no effect on lineage differentiation or the quality of the progeny [14, 15]. Viral transduction can also offer a long-term and stable production of the protein of interest. The most common vectors include retrovirus, lentivirus, baculovirus, and adeno-associated virus (AAV) [16]. Retrovirus leads to integration of the transgene into the host genome. While this results in a stable expression, it could also lead to insertional mutagenesis and activation of oncogenes [17]. Retrovirus is used when long-term protein production is desirable, such as treatment of genetic diseases. Lentivirus also enables stable transgene expression through integration into the genome.

Non-integrating lentiviral vectors have also been designed that can circumvent the problems associated with integration [18]. Baculovirus, on the other hand, is non-toxic; it neither replicates nor integrates into the host genome and is capable of transducing with high efficiency. Baculovirus can transduce adipose-derived stem cells (ASC) with 95 % efficiency and minimal toxicity [19]. Finally, AAV is one of the most promising vectors as it is non-pathogenic to humans and results in long-term gene expression. However, a large fraction of the human population have neutralizing antibodies against AAV, which drastically reduces their *in vivo* efficacy [20]. To circumvent the issue of activating oncogenes and to achieve targeted integration, Benabdallah et al. used zinc finger nuclease (ZFN) to add erythropoietin gene (Epo) into the chemokine (C-C motif) receptor-5 gene locus of MSC. ZFN was delivered to MSC using adenovirus while Epo was delivered using integrase-defective lentiviral vectors. The MSC derived from human BM, adipose tissue, and UCB was transduced with Epo by the ZFN-driven targeted gene addition. When these cells were injected into the peritoneum of non-obese diabetic severe combined immunodeficient interleukin-2Rγ null mice, the hematocrit levels rose from an average of 49 % to more than 60 % at day 10 [21]. This study clearly demonstrates the potential of site directed insertion (Figure 1A) compared to the conventional random integration using viral vectors. Site-directed integration can also be achieved using transcription activator-like effector nucleases (TALENS), and clustered regularly interspaced short palindromic repeats (CRISPR).

2.1.2. Non-viral transfection—While viral transductions have high efficiency, translation to the clinics has been hampered by high production cost and adverse immune reactions. Some viral vectors have limitations in the packaging capacity of exogenous DNA and the possibility of oncogene activation. Non-viral vectors, by contrast, are amenable to scale-up manufacturing, low in immune stimulation, and versatile with a wide array of design choices (Figure 1B). Use of non-viral gene delivery is also favorable for regenerative medicine, which requires only a transient expression. Unfortunately, MSC are difficult to transfect without affecting their viability, resulting in very low efficiencies. Current methods used to transfect MSC can be divided into physical methods and chemical methods. Physical methods include electroporation and nucleofection, both of which lead to significant cell death (~40 %) if not handled properly [22], and sonoporation [23]. In the case of electroporation an electrical pulse is used to transiently open the pores of the cells. This opening is used to drive the nucleic acid directly into the cytoplasm. Nucleofection also uses electrical pulse but the nucleic acid is directly driven into the nucleus of cells. Conversely, sonoporation uses mechanical vibration, such as ultrasound to increase the transport of nucleic acid into the cells by enhancing the permeability of the cell membrane. siRNA was successfully delivered into MSC using a combination of ultrasound and microbubbles (MBs) [23]. However, the acoustic intensity significantly affected the viability of the cells. Chemical methods include the use of lipid agents, polymeric carriers, dendrimers, and inorganic nanoparticles. Lipid and polymeric agents typically can transfect 2–35 % of MSC [24]. Dendrimers have shown great success in transfecting a wide variety of cell types but not MSC, typically achieving 10–17 % transfection efficiency only [25]. Inorganic nanoparticles mostly in combination with polycations have also been use to transfect MSC. Gold NP decorated with Jet-poly(ethylenimine) (PEI) reagent can condense DNA on the

surface to achieve a 2.5-fold increase in transfection efficiency over conventional Jet-PEI polyplexes [26]. Similarly, PEI attached to silica performed better than PEI alone, achieving 75 % transfection efficiency in human MSC [27]. The improvement is attributed to electrostatic interactions within the complex that helps stabilize the complex and reduce the size. More recently, Muroski and colleagues developed gold NP modified with Ku70 peptide using N-cysteine, which was able to transfect rat MSC with a transfection efficiency approaching 80 % [28]. This was achieved without affecting the viability and the properties of the MSC.

2.1.3. Applications of genetically modified MSC—Applications of genetic engineering of MSC can be divided into tissue engineering and drug delivery. In the case of tissue engineering and regenerative medicine, MSC are engineered mainly to increase their survival, retention, migration, and growth factor production. While, for drug delivery purposes, MSC are used to exogenously produce protein-based therapeutics to treat cancer or genetic diseases.

2.1.3.1 Tissue engineering and regenerative medicine: A majority of MSC often dies within the first few hours of *in vivo* delivery. To circumvent this issue, genetic engineering has been used to increase the survival of MSC *in vivo*. Overexpressing pro-survival, pro-angiogenic, or anti-apoptotic genes, such as protein kinase B (Akt1) [29], adrenomedullin, B-cell lymphoma-2 (Bcl-2) [30], and heme oxygenase-1 (HO-1) [31], has significantly increased the survival of MSC *in vivo*. Viral modification has also improved MSC' migratory behavior towards sites of injury, inflammation, and cancer through the overexpression of homing receptors, such as chemokine (C-X-C motif) receptor 4 (CXCR4) [32], and C-C chemokine receptor type-1 (CCR-1) [33]. MSC overexpressing CXCR4 home in greater numbers than unmodified MSC in the damaged infarct region of the myocardium. However, only a transient increase in CXCR4 expression is required for cells to reach the target site. With this in mind, Rejman and colleagues used cationic lipids and linear PEI to deliver CXCR4 mRNA. Since this bypasses the need to cross the nuclear envelope, transfection efficiencies of 80 % and 40 % were obtained using cationic lipids and linear PEI, respectively [34]. The mRNA was also detected within 30 min and persisted in the cytoplasm for about 9 days, which was more than enough time for the cells to reach the target site.

MSC have been most extensively used for treating bone-related diseases. Bone morphogenetic protein-2 (BMP2), transforming growth factor-β (TGF-β), latent membrane protein-1, insulin like growth factor-1 (IGF-1), and growth differentiation factor-5 are few of the proteins used to engineer MSC to enhance the generation of cartilage, bone, and tendon [35–37]. AAV-6 encoding BMP-2 and vascular endothelial growth factor (VEGF) have been used to induce bone formation and vasculogenesis [38]. However, most of the cells usually accumulate in the lung or the spleen. Therefore, to enhance the homing, MSC have been co-transduced with CXCR4 to increase migration; for example, MSC have been transduced with AAV to express runt-related transcription factor-2 (Runx-2) and CXCR4 to increase homing and mechanical strength of the bone in a murine osteoporotic mouse model [39]. MSC have also been engineered with retrovirus to express receptor activator of nuclear

factor-κB and CXCR4 to prevent bone loss [40]. The three important corner stones of tissue engineering are cells, scaffold, and bioactive factors. Recently, Brunger et al. attempted to combine all three factors by using poly-L-lysine (PLL) to immobilize lentivirus that encodes for TGF-β3 in a woven poly-ε-caprolactone (PCL) scaffold seeded with MSC [41]. Using a bioactive scaffold with properties similar to the native tissue to transduce MSC with TGF-β3 led to a sustained and robust cartilaginous extracellular matrix formation.

Non-viral approaches to deliver the required growth factors are the most rational approach to repair or induce bone formation since constitutive expression of genes is not imperative here. The abovementioned non-viral vectors are viable in delivering therapeutic genes, such as osterix [42], core-binding factor alpha 1 [43], and BMP2 [44], for bone regeneration [45]. Recently, cell sheets were engineered using LipofectamineR 2000 to produce anti-miR-138, a microRNA precursor family [46]. These cell sheets with high cell number and good cell-cell contact uses the scaffold-free tissue engineering approach and can be easily layered and attached to tissue beds. Moreover, the cell sheets enable the regeneration of massive bone with good vascularization.

MSC are also known to enhance cardiac repair through neovascularization, protecting myocardium from ischemic cell death. However, the bottleneck in effective translation of MSC for cardiac therapy lies in their poor survival, retention, and engraftment *in vivo*. Therefore, MSC have been engineered to enhance their survival using Akt1 [29], connexin43 [47], heat-shock protein 20 [48], Bcl-2 [30], and HO-1 [31], homing properties using CXCR4 [32] and CCR-1 [33], and differentiation into cardiomyocytes using murine hyperpolarization-activated cyclic nucleotide-gated 2 [9]. They have also been engineered to produce angiogenic factors using VEGF [49] and hepatocyte growth factor [50], and anti-inflammatory agents using interleukin-18 (IL-18) binding protein [51] to increase vascularization and reduce inflammation, respectively. For example, Yeh and colleagues used baculovirus to transduce ASC sheets to express VEGF [52]. The cell-cell junction in the cell sheets increased the survival and engraftment of the cell sheet. In addition, secretion of VEGF effectively arrested the loss of heart function, prevented myocardium fibrosis, reduced the infarct size, and increased the blood vessel formation.

2.1.3.2 Drug delivery for cancer and gene therapy: The inherent ability of MSC to migrate towards cancer cells makes them attractive as a cellular delivery vehicle for cancer therapy. MSC have been engineered to produce a wide variety of cancer therapeutic proteins, such as interleukins (IL-2 [12], IL-12 [53], IL-18 [54]), interferon-β [55], and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) [56]. MSC have also been engineered to produce prodrug-activating enzymes [57]. In this case, the genetically modified cells are followed by administration of the inactive prodrug locally, which is activated by the enzyme. This, in principle, would provoke the cell-killing action only at the site populated by MSC, minimizing the systemic toxicity. Some of the combination therapies that have shown success are: herpes simplex virus thymidine kinase with Ganciclovir, *E. coli* cytosine deaminase with 5-fluorocytosine, carboxylesterase with irinotecan, and cytochrome P450 with cyclophosphamide or ifosfamide [57]. Incorporating a tetracyclin-induced promoter to turn off the gene expression confers safety to the transplanted MSC. Delivering oncolytic virus is another popular approach taken for cancer

treatment. These viruses are designed to specifically replicate only in tumor cells and amplify only at the target site [58]. While there are quite a few oncolytic viruses in clinical trials, their translation is greatly hindered by the host immune response and inefficient viral distribution. MSC have been shown to be an effective carrier of oncolytic virus in treating ovarian tumors [59] and human hepatocellular carcinoma [60].

While engineered MSC have shown promise in treating cancer, monotherapy has not proven effective in treating highly heterogeneous cancer. Additionally, cancerous cells gain resistance to chemotherapy during their evolution, prompting the need for combination therapy. For example, TRAIL-engineered MSC delivered alongside temozolomide was more effective in treating glioblastomas than either of the individual therapies [61]. However, to make sure that both the drugs reach the tumor site simultaneously, MSC have been engineered to secrete CD20-specific single chain Fv antibody fused to TRAIL (scFvCD20-TRAIL). The combination was more efficient than delivering TRAIL-transduced MSC alone for treating non-Hodgkin's lymphoma because of the simultaneous inhibition of proliferation and induction of apoptosis in cancer cells [60].

MSC have been also used to treat monogenetic diseases, such as lysosomal storage disease or hemophilia, both of which require a high level of protein production afforded by viral transduction. Lentiviral transduction of MSC to produce high levels of biologically active β-glucuronidase led to successful cross-correction of lysosomal storage device *in vivo* [62–64]. Coutu et al. delivered engineered MSC to produce Factor IX (FIX) for treating hemophilia using a poly(lactic-co-glycolic acid) composite coated with biomineralized collagen-1 [65]. The long-term self-renewal and differentiation of MSC was promoted by the calcium phosphate ceramics, leading to therapeutic level production of FIX in hemophilic mice for over 12 weeks. For diabetes therapy, MSC have been transduced with AAV to increase expression of islet genes [8]. Pancreatic duodenal homeobox-1 has also been used to induce differentiation of MSC towards the β cell phenotype [66]. These studies demonstrate the potential of engineered MSC as a clinically relevant platform for treating various other genetic diseases.

Although genetically engineered MSC may be appealing for a myriad of diseases, the long-term safety of viral gene therapy remains a concern. As of now, only in diseases with a favorable benefit-to-risk ratio would such cell therapy be practical. Non-viral gene engineering may alleviate the safety concerns but its low transfection efficiency must be improved before it can rival viral methods.

2.2. Therapeutic Agent Incorporation

MSC have emerged as targeted drug delivery vehicles because of their 1) tissue homing ability; 2) low immunogenicity as they do not express histocompatibility complex class II molecule; and 3) anti-inflammatory property with the secreted growth factors and cytokines. As genetic engineering can only deliver protein drugs, NP delivery strategies can be applied to deliver non-peptidic drugs and therapeutic nucleic acids. Drug-loaded NP can be coupled with MSC through either cellular internalization or cell surface anchorage. As MSC migrate to the target tissue, the drug can be released in a local and sustained manner.

2.2.1 Loading via cellular internalization—Cellular internalization is one of the drug loading approaches. Drug-encapsulated NPs taken up *via* endocytosis have to escape the endolysosomal compartment to endow MSC with drug delivery property (Figure 2A). The endocytic and cytoplasmic localization processes are influenced by the composition, size, and surface charge of the NP [67, 68]. Various types of NPs, such as polymeric or liposomal NPs, with diameters ranging from tens to several hundred nanometers have been applied, and also with both negatively and positively charged NP. Poly(D,L-lactide) (PLA) and its analogues are FDA-approved, biodegradable drug delivery polymers that bear a weak anionic backbone under physiological conditions. Cellular internalization of PLA NPs is mainly through pinocytosis or clathrin-mediated endocytosis [69]. After endocytosis, the polymer undergoes a charge transition from negative to positive due to the acidic environment in the endosomal compartment and subsequently escapes from the endosome. Roger et al. loaded MSC with coumarin-6 encapsulated PLA NPs and showed that they did not affect the MSC viability even when the cells were treated with a dosage of 200 μg/mL NPs. The endocytosis of PLA NPs by MSC was time and concentration dependent, and very efficient [70]. Another PLA analogue, poly(D,L-lactic acid-co-α,β-malic acid) (PLMA), has also been used for MSC loading. PLMA NPs composed of iron oxide and the dye, FITC, was designed and used as a bifunctional contrast agent for MSC tracking under magnetic resonance and fluorescent visualizations. These NPs were taken up by MSC without affecting their viability and differentiation capabilities into adipocytes and osteocytes [71]. Similar results were reported for the negatively charged NP, mesoporous silica NP [72].

NPs with cationic surfaces have also been used to improve the cellular uptake by MSC [73–75]. Cationic polymers that have been widely used for non-viral gene delivery for MSC [76–78], such as PLL and PEI, are also efficient for drug loading applications. Kostura and colleagues coated superparamagnetic iron oxide (SPIO) NPs with PLL and showed that PLL-coating could significantly enhance the cell uptake by MSC. The percentage of cells loaded with NPs reached nearly 100 %, whereas no significant uptake was observed when MSC were treated with unmodified SPIO NPs [79]. The size of the coated PLL was further optimized: when SPIO NPs were coated with a moderate-sized PLL (MW 388 kDa), more than 92 % of cell loading efficiency was achieved, whereas only 80 % was observed when lysine monomer was used [80]. In addition to PLL, a series of low molecular PEI (MW 2 kDa)-SPIO NPs for MSC loading was designed and optimized. These PEI-SPIO NPs with sizes ranging from 20 to 80 nm also showed high loading efficiency (93±2 %) and did not have significant toxicity on MSC [81].

In addition to polymeric NPs, liposomal NPs have been used to deliver therapeutic cargo to MSC [70, 82, 83]. They can also be taken up by MSC through endocytosis and release their cargos intracellularly by fusing to the endosomal membrane. In contrast to polymeric NPs, high doses of liposomal NPs are usually required to achieve effective cell loading efficiency, which leads to significant cytotoxicity [70, 82]. In addition, small therapeutic cargos can be directly loaded onto MSC without any carrier encapsulation. Pascucci et al. used murine MSC as a vehicle to deliver a chemotherapy drug, paclitaxel (PTX). They demonstrated that MSC were able to take up and release PTX although the drug inhibited the cells'

proliferation ability. In spite of this, *in vitro* results showed that the released products from the PTX-primed MSC could kill cancer cells [84].

The success of non-viral transfection has inspired researchers in the field to apply the same concepts for loading MSC with therapeutic agents and use them as drug vehicles. The abovementioned studies show that MSC are capable of internalizing the therapeutic agent-loaded NPs or the drug directly. Generally, polymeric NPs are more efficient for drug loading compared to liposomal NPs [82] and a positive NP surface charge enhances the cell loading efficiency [75]. Beside these strategies, receptor-mediated endocytosis is also a possible way to enhance the cell loading efficiency. Decorating NPs with antibody against MSC surface antigen CD90 could improve the kinetics of NP internalization [85].

2.2.2 Anchoring via lipid fusion—As explained in 2.2.1., liposomal NP has been used to load MSC with therapeutic cargos. Figure 2B shows the basic mechanisms of a non-covalent strategy to modify the MSC surface with therapeutic agents. When NPs interact with the MSC surface, they undergo membrane fusion and the cargos are transferred and anchored on the cell. As a result, a synthetic “receptor” could be created on the cell surface. Dutta and colleagues demonstrated this strategy on fibroblasts. Various functional groups or fluorescent dyes can be transferred from the liposomes onto cells [86]. A similar approach has been applied to MSC for surface modification. Biotin-modified liposome was used to present biotin on the cell surface, which serves as the bridge for subsequent ligand immobilization *via* the biotin-streptavidin interaction [87].

2.2.3 Mechanisms and strategies to control the release—For MSC that are primed with therapeutic cargos, exocytosis and simple diffusion are the two major routes of cargo release. Exocytosis is a removal process that varies with NP type and size as well as cell type. Cells can release half of the internalized cargos between 30 minutes to few hours [88, 89]. In a fluorescent dye transfer assay, more than 85 % of the internalized dye was transferred from MSC to the co-cultured cancer cells through exocytosis [90]. In contrast, if the cargos were released from the endosomal compartments, which are typically governed by a combination of matrix degradation- and diffusion-controlled release mechanisms, the drug release profile from the MSC would be slower and more prolonged. By combining the exocytotic and endosomal release mechanisms, a biphasic profile may fit the release kinetics of therapeutic agent-primed MSC. SPIO-loaded MSC studies have revealed this phenomenon. Here the cells’ iron content dropped significantly in the beginning stage and cells released half of the iron within the first two days, which may be dominated by exocytosis route. After that, relatively slow diffusion kinetics could be observed as the iron was totally removed by two weeks [82, 91]. To control the diffusion kinetics, a multidrug resistance-1 gene was used to generate P-glycoprotein 1, which could actively pump out the loaded drug from MSC cytosol [92]. While this modification can tune the release profile, it also raises safety concerns, which will be discussed in section 3.

For the cargos anchored on the cell surface, the release profile is regulated by the dissociation constant between the “synthetic” receptor and its ligand (the therapeutic cargos). For example, if the MSC surface is modified with biotin and then the cargo is loaded through biotin-avidin interaction, one may expect a very slow release profile since

the affinity between biotin and avidin is very strong (the dissociation constant is ~10^{-16} M) [93].

2.2.4 Applications of therapeutic agent loaded MSC—The MSC loaded with therapeutic agents have been used for tissue regeneration and cancer therapeutics. For tissue regeneration, it is essential that the multi-lineage differentiation potential of MSC is not affected by the uptake or fusion of NPs. Most of the NPs have been reported not to influence MSC' differentiation capability with the exception of titanium and cobalt/chromium/molybdenum NPs [94]. As a result, NPs, especially, SPIO-containing NPs, have been incorporated with MSC for repairing a broad spectrum of tissue types. SPIO-containing NPs are generally used for improving MSC' homing ability. With the assistance of external magnetism, SPIO-primed MSC localize on the injured tissue site. Figure 2C shows a typical example of the magnetic force-assisted tissue localization of SPIO-primed MSC. Luciferase-expressing MSC either with or without SPIO-loading was transplanted into a rat with muscle injury. Subsequently, a 3T external magnetic force was applied on the site of injury, and a significant increase of MSC localization in the case of SPIO-primed MSC was observed [95]. Similar approaches have been applied to other types of tissue injuries including vascular [82, 83] and cartilage repairs [96]. A preclinical arterial injury model has been used for magnetic-assisted MSC delivery. The femoral artery injury on rabbit was created and treated with SPIO-primed MSC and an external magnet guide. After treatment for three weeks, a significant reduction of restenosis was seen without losing the level of re-endothelialization (Figure 2D) [82].

Additionally, NP-loaded MSC are also used for cancer therapy. By using MSC' homing properties, cancer drugs can be precisely delivered to the tumor site, and drug-loaded MSC can release the drug *via* exocytosis and diffusion. Exosomes from drug-loaded MSC have also shown to effectively kill cancer cells; for example, exosomes collected from PTX-loaded MSC were successful in inhibiting the growth of pancreatic adenocarcinoma cells *in vitro* [84]. Furthermore, Huang et al. have conducted an *in vitro* transwell assay to demonstrate that the tumor tropism of MSC is not affected when MSC are loaded with NPs [97]. They have also showed in a murine glioblastoma model that contrast agent-primed MSC can cross the blood-brain barrier to the tumor site. The result demonstrated that the MSC-treated group showed 5.2-fold increase of contrast agent accumulation on tumor compared with the group treated with only NPs (Figure 2E) [97]. This indicates that drug-loaded MSC may be more advantageous and efficient compared with the conventional cancer therapeutics.

2.3. Transient Cell Surface Modifications

Although genetic modification and therapeutic agent incorporation techniques have been used to improve MSC' native properties to increase the therapeutic efficacy, the major limitation of using MSC as cellular therapeutic carriers is the inability to deliver a therapeutically effective dosage to the desired site. Despite the fact that the homing property of MSC to inflammatory and tumor sites has been well documented [98], the exact mechanism of the homing property of endogenous and exogenous MSC has still not been elucidated. However, it is accepted that several mediators including chemokines [30, 99],

cell adhesion molecules [100, 101], matrix metalloproteinase [102, 103], and cytokines [104, 105] play a vital role. Nevertheless, relying solely on the homing property of MSC is insufficient to achieve significant therapeutic cell dosage at the disease site as MSC often lack the homing receptors expressed on hematopoietic stem cells (HSC) or leukocytes, and hence are not as efficient. Moreover, *ex vivo* handling (passage frequency, confluency, and the microenvironment of the culture conditions) of the cells can cause them to lose their homing ability [103, 106]. Furthermore, upon systemic administration, most MSC become filtered and accumulate in the lung, liver, and spleen before they are eliminated from the body [107, 108]. Consequently, approximately less than 1 % of the injected MSC reach the target site [107, 109], which would not be enough to drive the therapeutic effect *via* paracrine signaling. Therefore, several approaches have been used to enhance the therapeutic dosage of MSC. One approach might be to inject more of them to ensure that an effective number of cells reach the target site. However, direct injection (intravascular or intra-arterial injection) of a large number of cells results in complications attributed to microembolism, which may lead to vascular obstruction, stroke, and potentially, death [110, 111]. Furthermore, compared with the commonly used animal models, humans would require larger quantities of MSC, the production of which may require excessive expansion that might diminish their pluripotency [106]. Therefore, there is a limit to dose escalation for improved therapeutic outcomes and a need to establish safe administration protocol for high cell numbers.

A better approach would be to augment the homing efficiency of MSC by using surface modification techniques to introduce targeting moieties: 1) by exploiting the HSC' and leukocyte's transmigration process to target activated endothelium of the therapeutic site; 2) by using chemical or non-covalent interactions; or 3) by exploiting biospecific recognition (antigen-antibody or ligand-receptor). These techniques are not only versatile and economic, but also potentially safer than that of genetic modification and therapeutic agent incorporation because they avoid the long-term safety and regulatory issues, and toxicity issues, respectively. Because transmigration of transplanted MSC to the activated endothelium occurs between 60 and 120 minutes [112], the expression of transient homing molecules would enhance the targeting efficiency of MSC to the target site. Additionally, surface modification techniques can enhance the adhesion and engraftment of MSC without compromising their viability, adhesion, multipotency, proliferation, and differentiation potentials [87, 113–115]. In the next section, we will discuss three different surface modification strategies that improve the cell homing property (Figure 3), and thereby potentially avoid the risk and need to inject more cells.

2.3.1. Enzymatic modification—The most popular method for enhancing the migratory behavior of MSC is to functionalize MSC with adhesion ligands, such as hematopoietic cell E-/L-selectin ligand (HCELL) and P-selectin glycoprotein ligand-1 (PSGL-1), which may be absent, insufficiently expressed naturally [116], and/or lost during the expansion of cells *ex vivo* [106]. These ligands mediate adhesive interactions (e.g., tethering and rolling) due to the constitutive expression of selectins on bone marrow and inflamed tissues [117]. For this purpose, the most characterized selectin ligands have been widely used to engineer transmembrane glycoproteins on HSC and MSC. This concept originates from the

endogenous lymphocyte infiltration from blood to inflamed tissues, where the overexpression of cell adhesion receptors mediate the cell rolling process *via* the selectin-dependent pathways [118]. Cell rolling enables the leukocyte to slow down and interact with the locally secreted chemokines, which subsequently leads to the activation of integrins, followed by firm adhesion and invasion of leukocytes within the inflamed vasculature [118]. Within this cascade, the rolling step initiates the leukocyte extravasation and plays a crucial role in the subsequent adhesion and transmigration processes [87]. Several immunoprecipitation and mutation studies have demonstrated that the absence, modification, or blocking of the effector molecules associated with the cell rolling response prevents firm adhesion under shear stress *in vivo* [100, 104].

Xia and colleagues first tested this method by enzymatically modifying the surface of human umbilical cord blood (CB) CD34+ cells with selectin-binding motifs (α-1,3-fucose) to improve their homing to the bone marrow under shear stress. For selectin to bind to its cell surface ligand, it first needs to be fucosylated to form glycan determinants (epitopes). Therefore, to transiently display fucose residues on the surface glycans, fucosyltransferase VI (FTVI) and the fucose donor substrate, guanosine diphosphate fucose, were used [119]. This modification generated α-1,3-fucose-functionalized CB CD34+ cells, which led to an increased rolling response on the E- or P-selectin-immobilized plates at 0.5 dyn/cm^2 by 1.9- and 2.9-fold compared to the unmodified MSC, respectively [119]. *In vivo* results also demonstrated an increase in bone marrow engraftment of systemically administered CB cells in immunocompromised mice.

Extending this concept one step further, Sackstein et al. engineered MSC, which naturally lack the expression of E-selectin ligands, to display selectin-binding motifs (α-2,3-sialic acid and α-1,3-fucose) on the endogenous surface glycans, which are carbohydrate polymers on a glycoprotein. The resulting sialofucosylated glycan epitope on CD44 is known as Sialyl-Lewis X (SLe^X), the selectin-binding carbohydrate motif, which is an active site for HCELL and PSGL-1 expressed on human hematopoietic stem/progenitor cells (HSPC) and leukocytes, respectively. Enzymatic modification of endogenous CD44 into its sialofucosylated glycoform (HCELL, as represented in Figure 3) enables any cells expressing CD44 to bind firmly to E- and L-selectin expressing cells without going through conventional multistep chemical synthesis [113].

2.3.2 Chemical modification—Enzymatic modification of the cell surface glycan is limited to the glycoproteins that already exist on the cell surface and may potentially affect other critical cell surface molecules by non-specific interactions. In contrast, chemical modifications enable MSC to present several targeting moieties, including ligands [116], peptides [114], and polymers [120], *via* covalent bioconjugation method. Sarkar et al. covalently modified MSC to transiently decorate SLe^X by first using N-hydroxy-succinimide (NHS) chemistry, and then subsequently using the biotin-streptavidin interaction. This targeting moiety, SLe^X, can be immobilized on the surface of MSC by first modifying the amine groups on the cell membrane to biotin-NHS and subsequently adding streptavidin to introduce the biotin binding sites. Then, P-selectin-binding MSC is obtained by mixing and incubating them with either biotinylated lipid vesicles [87] or a polymer construct (~30nm) [116], containing SLe^X determinants. Biotinylated lipid vesicles

intercalate and fuse to the cell membrane of MSC and display biotin on its surface, offering a docking site for subsequent conjugation with biotinylated SLe^X after treating MSC with streptavidin. MSC modified with SLe^X using this method resulted in promoted cell rolling, adhesion, and migration [121].

This versatile and simple technique can be used to display any targeting moiety without affecting the microenvironment of the modified MSC, which is controlled by their paracrine factors (such as stromal cell-derived factor-1 (SDF-1) and IGF-1) [116]. This conventional chemical conjugation technique has also been recently adapted to present bone-targeting protein conjugated polymer on MSC [120]. Moreover, chemical modification offers a relatively stable covalent binding of the targeting molecules on MSC – at least 6 hours without the functionalized peptide getting internalized – without affecting the cells' viability, proliferation, and multipotency [114].

2.3.3. Non-covalent modification

2.3.3.1. Antibody conjugation: Another clinically attractive approach for improving the specific homing of MSC is antibody conjugation, due to antibodies' high affinity towards the overexpressed antigens on the target site. This strategy has been especially successful in cancer immunotherapies by driving the immune system against cancer cells using a bispecific antibody, which simultaneously binds to two different antigens, or an engineered receptor known as chimeric antigen receptor, where the antibody-binding domain is connected to the T-cell activating domains [122]. As exploited in T-cells, antibody conjugation to the stem cells has also been achieved to localize MSC at the therapeutic target. This is realized by using palmitated proteins G [123–125] or A [126], or a bispecific antibody.

Engineering the cell surface with palmitated proteins, which act as hydrophobic anchors, allows the subsequent conjugation of potentially any antibody with an accessible Fc cassette or a protein fused to this domain [127]. Chen and colleagues optimized the protocol [128] from Kim and Peacock [126] and first established a concept called "protein painting." This entails the intercalation of palmitated proteins on the cell membrane as a pre-coating step to bind antibodies to cell surfaces while neither compromising their binding affinity to specific antigens [126, 128] nor the viability and differentiation potentials of the cellular carriers [123, 129]. This simple and versatile method was first used to home chondrocytes for cartilage repair in the rabbit explant model by binding antibody targeting cartilage extracellular matrix [123].

Bispecific antibodies offer an interesting targeting strategy as they can bind to two different antigens to guide stem cells to the desired tissues and promote stem cell retention as well. This strategy was successful in homing murine stem cells expressing c-kit to the murine injured myocardial cells overexpressing vascular cell adhesion molecule-1 (VCAM-1) using anti-c-kit and anti-VCAM-1 bispecific antibodies [130]. Similarly, this method was applied to repair the function of the infarcted heart by arming human HSCs with bispecific antibodies consisted of anti-human CD45 and anti-rat myosin light chain antibodies [131]. This technique was adapted by Deng et al. to modify MSC with bispecific antibodies [132]. However, bispecific antibodies have not been widely used with MSC, likely due to the lack

of a specific cell surface marker [133]. Moreover, the instability and the high production cost of generating clinical-grade bispecific antibodies *via* heteroconjugation of two antibodies (using chemical, genetic, or hybridoma methods) are the limiting factors for translating this modality to clinics.

2.3.3.2. Peptide conjugation: Although an antibody provides a specific affinity towards its antigen, it is expensive to produce on a large scale with high purity. Peptide conjugation, which has also been widely used in preclinical studies, may be a more attractive strategy. Peptide production is inexpensive, relatively simple, and scalable with high purity. Furthermore, using the phage display technique, the peptide sequence with the highest binding affinity to the target tissue can be tested and further optimized. Along with the chemical coupling method, peptide conjugation can be realized by employing hydrophobic interactions where the homing peptide conjugated to lipid groups (palmitate derivatives) [134, 135] are integrated on the cell membrane composed of a lipid bilayer. With coating of palmitated proteins or their derivatives, any type of cell (MSC, red blood cells, chondrocytes, etc. [166]) can be conjugated with peptide to home to the specific target.

2.3.4. Applications of surface-modified MSC—Surface modification techniques introduce targeting moieties, which can bind to the proteins overexpressed in the diseased or damaged tissue *via* covalent or non-covalent interactions. Hence, the therapeutic dosage of MSC trafficking to the target is increased and the subsequent therapeutic efficacy may be enhanced. Collectively, these methodologies that modify exogenous MSC demonstrate the potential for engineered MSC to be used in regenerative medicine and drug delivery applications without significantly affecting the cells' viability, multipotency, and differential potentials. Surface-modified MSC have been used for three major applications: bone regeneration, repair of inflamed tissues, and treatment of myocardial infarction.

2.3.4.1. Bone regeneration: Although MSC differentiates into osteoblasts, naturally they do not migrate to bone. Therefore, only by engineering MSC can they be targeted to bone for bone regeneration. Because of the constitutive expression of selectins on bone marrow [117], modified MSC can target the bone marrow using the enzymatic modification technique. The forced glycosylation of the membrane surface glycans confers HCELL on MSC. As a result, firm adhesion of glycosylated MSC to the microvascular vessel in bone marrow was achieved under hemodynamic shear stresses of up to 30 dyn/cm^2 [113]. Intravenous injection of HCELL-expressing human MSC into immunocompromised mice also significantly enhanced the homing response to the calvaria of the mice, as compared to controls. Likewise, HCELL facilitates the migration of other cells; for instance, HCELL-expressing murine HSPC resulted in more than 3- fold higher homing to the bone marrow compared to the unmodified control [136]. HCELL also plays a role in mediating the metastasis of colon cancer and circulating tumor cells [137, 138]. Taken together, exploiting the tropisms to bone, MSC can be decorated with HCELL by enhancing the transmigration of MSC to the bone *via* the selectin-mediated cell adhesion pathway.

A recent paper published by D'Souza et al. combined a covalent conjugation method with polymer-based engineering to enhance the homing property of MSC for treating bone injury sites [120]. Atom transfer radical polymerization was used to synthesize a bone-targeting

moiety that contains alendronate (Ale). This drug has high affinity to bone and is known to decelerate bone resorption by inducing the apoptosis of osteoclasts, thereby shifting the bone remodeling balance toward osteogenesis by osteoblasts [139]. MSC armed with bone-targeting polymer can home to hydroxyapatite crystals and rodent bone fragments, suggesting their potential for bone regeneration therapy. By contrast, other studies have used the biospecific recognition of the α4β1 integrin receptor on the MSC surface and a synthetic peptide (LLP2A) mimicking the ligand against the activated integrin to augment osteogenesis [140, 141]. A hybrid compound of Ale and LLP2A was covalently coupled (LLP2A-Ale) and conjugated to MSC, which allowed them to navigate and tether to bone and promote osteoblast differentiation *in vitro* and *in vivo*. These studies demonstrate that functionalizing these dual targeting (MSC and bone) polymers or peptide-Ale hybrids on MSC can be used to treat bone fracture healing and bone degenerative diseases, such as osteoporosis.

2.3.4.2. Inflammatory disease treatment: Modified MSC are not only exceptional in targeting bone marrow, but also in navigating to inflamed tissues expressing selectin [117] using the chemical modification [87] and antibody conjugation [124] approaches. Functionalized MSC with SLe^X using biotinylated lipid vesicles exhibited the rolling activity on the P-selectin surface under a shear stress of 0.5 dyn/cm^2 without affecting the multilineage differentiation potentials, viability, proliferation, and adhesion kinetics of MSC [87]. Similarly, using an optimized biotinylated polymer construct resulted in a sturdier cell rolling response under a shear stress of up to 2 dyn/cm^2 on the P-selectin substrate. An *in vivo* study also indicated that the imaging agent-labeled, SLe^x-modified MSC navigate towards the inflamed ear 56 % better than the negative control [116]. Exploiting the same NHS chemistry, another study used a flexible linker to present E-selectin-binding peptides on MSC for promoting firm adhesion and the following rolling processes on E-selectin substrates under a shear stress of up to 10 dyn/cm^2 [114]. Therefore, by exploiting the leukocyte extravasation *via* the selectin-mediated rolling response, this simple, robust, and versatile strategy promotes targeting of MSC to the sites of inflammation upon systemic injection.

Ko and colleagues tailored MSC to the inflammatory sites by anchoring antibodies of the upregulated cell adhesion molecules, such as intercellular adhesion molecule-1 (ICAM-1) [124], the VCAM-1, and the mucosal vascular addressin cell adhesion molecule (MAdCAM) [125]. MSC coated with the ICAM-1 antibodies substantially promoted their adhesion to the activated endothelial cells *in vitro* [124]. Moreover, the homing abilities of the MAdCAM or VCAM-1 directing MSC were demonstrated by the prolonged survival (approximately 2.3-fold compared to unmodified MSC) of the treatment group injected with the MAdCAM antibody-coated MSC tested in the *in vivo* model of inflammatory bowel disease. Efficient localization to colon and suppression of regulatory T cells was achieved with the VCAM-1 antibody-coated MSC *in vivo*. Interestingly, this study demonstrates that better localization of MSC does not always result in increased therapeutic efficacy, which agrees with the latest study that the therapeutic outcome does not correlate with the engraftment efficiency [142]. This will be further discussed in section 3.

2.3.4.3. Myocardial infarction repair: Employing molecular recognition techniques, such as surface-modified MSC with bispecific antibody [132] or peptide conjugation [134, 135], Deng et al. improved the homing and therapeutic efficacy of MSC in a murine myocardial infarct model by combining the bispecific antibodies (anti-mouse CD29 and anti-myosin light chain antibody) with ultrasound-mediated MBs. The combination approach significantly increased the homing of MSC at the target site and reduced the fibrosis density compared with the unmodified MSC or MSC coated with bispecific antibodies only [132]. This synergistic effect was due to the enhanced migration of the MSC, which changed their microenvironment by activating the STAT-mediated signaling pathway. The increased homing also improved the therapeutic efficiency.

MSC have also been presented with various palmitated-ischemia-homing peptides in the rodent myocardial infarction model using the transient “protein painting” method, as explained in 2.3.3.1. By doing so, increased localization of peptide-coated MSC was achieved at the diseased site up to 10 times more than the unmodified cells [134, 135]. These results demonstrated the enhanced localization of modified MSC with targeting peptides *in vivo*; however, the stability of the peptide coated on MSC decreased to 70 % after 2 hours of incubation at 37 °C [135]. This may be due to internalization of the peptides anchored on the cell surface *via* endocytosis or proteolysis. Moreover, none of these studies tested whether the weak hydrophobic interaction between the cell membrane and the homing peptide is maintained under physiologically relevant shear stress. Hence, Lo et al. adapted this “painting” method to present a fusion protein of PSGL-1 N-terminal peptide and human IgG1 on MSC to assess whether they can attach to the selectin-expressing endothelial cell layers under flow. Without affecting the vital phenotypes of MSC, the tethering, rolling, and retention under flow (up to 10 dyn/cm^2) were significantly improved compared to the unmodified MSC [115]. The stability of the peptide could not be compared with the previous studies due to different incubation temperatures, but 66 % of the MSC complexed with the fusion proteins were maintained after 24 hours of incubation at the room temperature, demonstrating a transient expression of the fusion protein.

Potentially, these methods could generate engineered MSC to target specific tissues for regeneration or drug delivery applications as multiple peptides and antibodies can be coated onto the cell membrane.

3. Challenges and Future Directions

As discussed in the previous sections and summarized in Table 1 with selected examples of different engineering strategies, they offer new modalities and opportunities for MSC-based therapy and tissue regeneration. Each technique has its own benefits and drawbacks, as summarized in Table 2, and the challenges of these strategies for translation are still being defined; they are neither insignificant nor insurmountable. However, they would be worth addressing because of the amazing potential that MSC offer.

One of the major barriers limiting the clinical translation of allogeneic MSC is the absence of a biomarker to evaluate the quality of MSC from different donors with varying ages, sources, and genetically diverse backgrounds. Moreover, the lack of standardized protocols

to extract, culture, and prepare MSC has led to large variability in their properties. Therefore, engineering these heterogeneous populations of MSC to begin with may affect the safety profile and lead to unpredictable clinical outcomes. Hence, a more stringent approach, such as automating the *ex vivo* expansion process, may be crucial for the standardized use of MSC in the future. Also, it is imperative to identify the relevant biomarkers and establish a screening/monitoring method to evaluate, quantify, and predict the clinical effectiveness of MSC for the further development of MSC-based therapies.

Although viral modification is the most efficient and prominent engineering modality for enhancing the therapeutic functions of MSC, its long-term safety concerns, including eliciting an immune response and activating oncogenes, have hampered the clinical translatability of this approach. It is also limited in the size and number of genes that can be simultaneously delivered. Yet, with advancement in the field of genetic engineering, molecular-editing tools, such as TALENS and CRISPR, may be exploited to improve safety and efficiency of inserting or expressing large or multiple genes. Non-viral gene therapy, on the other hand, is severely limited by the low transfection efficiency. A concerted effort to optimize all aspects of non-viral gene transfer ranging from construction of efficient plasmids to design of stimulus-responsive gene carriers and to improved transfection protocol is required. For instance, microfluidics may be used as a rapid, highly reproducible, and scalable platform to transfect MSC with uniform properties and high transfection efficiency. Furthermore, combinatorial and high throughput approaches can be used to screen carrier design and formulation to improve the therapeutic performance of MSC.

NP incorporation strategies may have detrimental effects on MSC. For instance, proliferation of MSC is inhibited by PTX [84] and its viability is affected by other chemotherapeutic drugs, such as vincristine and 5-fluorouracil [143, 144]. Hence, MSC is not a universal platform for delivering any therapeutic agents. Moreover, for loading therapeutic agent on NPs, it would be essential to identify whether the properties of MSC are adversely affected because some NP carriers may affect the multipotency of MSC [94]. Furthermore, as a delivery platform, cargo release kinetics needs to be optimized. Since MSC release the cargo through exocytosis and diffusion, the kinetics of drug release from the MSC platform could be predicted if we can estimate how fast and how many NPs can cross the intracellular barriers and unload the cargo into the cytoplasm of MSC. A fluorescence resonance energy transfer-based approach using quantum dots and their fluorescent pairs might be suitable to examine the release kinetics of NPs before engineering MSC [145, 146]. For loading therapeutic agents through membrane anchoring, the release kinetics is controlled by the dissociation between the drug and its receptor, so it may be easier to predict the kinetics using techniques established for studying ligand-receptor interactions. However, researchers using this approach would face two other issues, drug degradation and drug immunogenicity. Because the drug in this approach is not protected, enzymatic or hydrolytic degradation may lead to inactivation of the therapeutic agent, which may also elicit an immune response.

Although investigations on the surface modification of MSC have not revealed serious compromise of their functions so far, potential danger remains until long-term and detailed *in vivo* studies are conducted. Other than biological concerns, surface-modified MSC will be

directly exposed to a shear stress environment, conditions that may lead to aggregation, internalization, and shedding of the active moieties. Variable expression of the targeting molecules may confound the homing and therapeutic efficacy of the engineered MSC, which would significantly hamper the clinical translation of this approach. Therefore, surface-functionalized MSC need to be rigorously tested under clinically relevant conditions *in vitro* and tested with large sample size to assess their impact *in vivo*. Furthermore, as homing and healing are both orchestrated by multiple effector molecules, improving the localization of modified MSC to the target neither guarantees enhanced MSC transmigration nor promises higher therapeutic outcome [125]. For example, if the cells lack adhesion molecules, chemokine receptors, or other key associated molecules for transmigration and invasion, presenting MSC with targeting moieties may not be sufficient to enhance the homing or engraftment processes [119, 124]. Nevertheless, surface modification approach bypasses many risks associated with multiple MSC administrations, such as activating the adaptive immunity. The variability in clinical responses may also be related to the presence or absence of appropriate cues in the host's microenvironment to maintain the bioactivity of MSC. Therefore, providing a suitable niche for implanted MSC at the affected tissue has become a paramount factor to improve engraftment, which leads to extending the therapeutic outcome and reducing the effective therapeutic dosage. There is ample evidence that delivering pre-seeded cells in a polymeric scaffold may promote their engraftment, survival, and tissue regeneration [147]. These biodegradable scaffolds, which are designed to mimic the native ECM, also provide structural and mechanical support for better cell engraftment at the target site. Although details of the scaffold-based strategies are not covered herein, they have been discussed in other excellent reviews [148], [147], and [149]. Therefore, future studies need to also focus on inducing multitudes of biochemical and physical cues to affect the microenvironment of the MSC, which may enhance the desired therapeutic efficacy. For instance, combining both the targeting and therapeutic agents (i.e. NPs, peptide drugs, exogenous genes etc.) on MSC and deliver them *via* an optimally designed implantable scaffold (with optimized porosity, biocompatibility, degradability, and mechanical and biochemical properties) may be a fruitful direction.

4. Concluding Remarks

Owing to their remarkable self-renewal, multipotent, immunosuppressive, and homing properties, MSC are attractive candidates for cell-based therapy in their own right. They can regenerate damaged tissues, deliver therapeutic gene(s)/agent(s), and exert paracrine effects, which lead to recruiting effector cells necessary for regenerative therapeutics and disease treatments. Recently, researchers have sought to further enhance these therapeutic properties of MSC. Through various approaches, MSC can be engineered to express exogenous genes, incorporate therapeutic cargos, or present targeting moieties to enhance their survival, homing, and therapeutic efficacy. These strategies offer therapeutic dosages of MSC and active agents at the target site, circumventing the need for repetitive systemic administration. However, to maintain or augment the tropism of engineered MSC to inflamed and cancerous sites for regeneration and treatment purposes, respectively, more detailed studies need to be conducted to better understand the behavior of native MSC in homing, healing, and differentiation. In addition, to be clinically translatable, optimal *ex vivo* handling and

preservation protocols and standards for quality and safety profile of MSC need to be established. The usage of MSC for personalized medicine and tissue repair has been promising. As new and unexpected properties of MSC are discovered, as has been the case in the past decade, MSC-based therapy is likely to become even more prominent. The engineering strategies described in this review to modify MSC will further enhance their functionality and potency. It is by no means an easy task to overcome the scientific hurdles of MSC modification, but it is a challenge worthy of scientific undertaking given the potential reward.

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Abbreviations

MSC	Mesenchymal stem cells
BM	Bone marrow
UC	Umbilical cord
iPSC	Induced pluripotent stem cells
UC-MSC	UC-derived MSC
BM-MSC	BM-derived MSC
NP	Nanoparticle
AAV	Adeno-associated virus
ASC	Adipose-derived stem cells
ZFN	Zinc finger nuclease
Epo	Erythropoietin gene
TALENS	Transcription activator-like effector nucleases
CRISPR	Clustered regularly interspaced short palindromic repeats
MBs	Microbubbles
PEI	Poly(ethylenimine)
Akt1	Protein kinase B
Bcl-2	B-cell lymphoma-2
HO-1	Heme oxygenase-1
CXCR4	Chemokine (C-X-C motif) receptor 4
CCR-1	C-C chemokine receptor type-1
BMP2	Bone morphogenetic protein-2

TGF-β	Transforming growth factor-β
IGF-1	Insulin like growth factor-1
VEGF	Vascular endothelial growth factor
Runx-2	Runt-related transcription factor-2
PLL	Poly-L-lysine
PCL	Poly-ε-caprolactone
ILs	Interleukins
TRAIL	Tumor necrosis factor-related apoptosis-inducing ligand
scFvCD20-TRAIL	CD20-specific single chain Fv antibody TRAIL fusion
FIX	Factor IX
PLA	Poly(D,L-lactide)
PLMA	Poly(D,L-lactic acid-co-α,β-malic acid)
SPIO	Superparamagnetic iron oxide
PTX	Paclitaxel
HSC	Hematopoietic stem cells
HCELL	Hematopoietic cell E-/L-selectin ligand
PSGL-1	P-selectin glycoprotein ligand-1
CB	Cord blood
FTVI	Fucosyltransferase VI
SLe^X	Sialyl-Lewis X
HSPC	Hematopoietic stem/progenitor cells
NHS	N-hydroxy-succinimide
SDF-1	Stromal cell-derived factor-1
VCAM-1	Vascular cell adhesion molecule-1
Ale	Alendronate
ICAM-1	Intercellular adhesion molecule-1
MAdCAM	Mucosal vascular addressin cell adhesion molecule

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Highlights

- Engineered mesenchymal stem cells (MSC) augment their innate properties.
- Genetically engineered MSC enhance their therapeutic outcome.
- Therapeutic agent-loaded MSC serve as effective drug delivery systems.
- Surfaced-modified MSC home to target tissue with high efficiency.

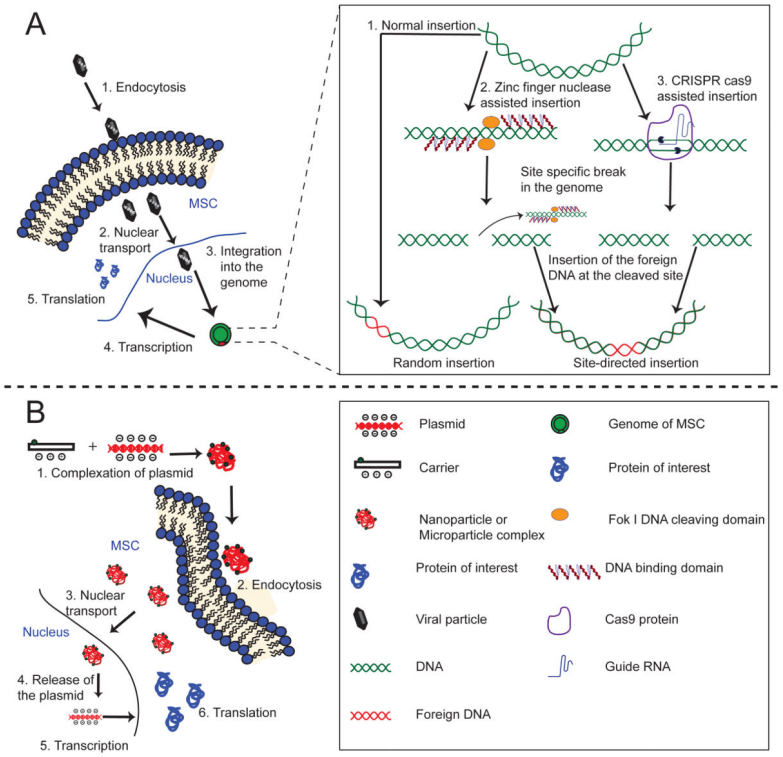

Figure 1.

Schematic diagram of the mechanisms of viral and non-viral gene delivery. A: Viral gene delivery. In this case, the insertion of the target gene into the host genome is usually random. The insertion process can be ameliorated by cutting/inserting specific sequence using ZFN or CRISPR/Cas9 system. B: Non-viral gene delivery. In this case, the plasmid does not integrate into the genome. Figure not drawn to scale.

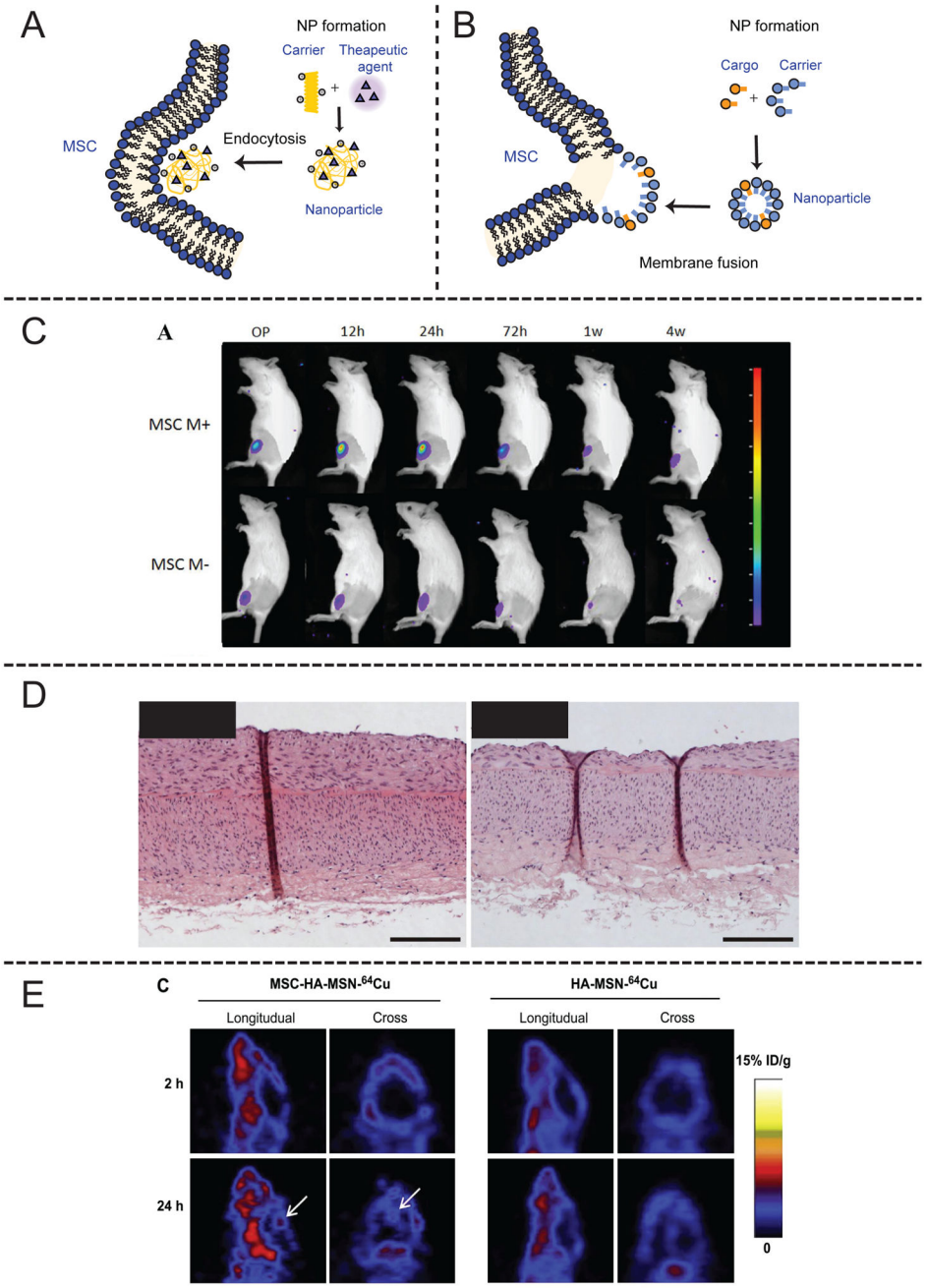

Figure 2.

Schematic diagram of the selective mechanisms of MSC to uptake multifunctional NPs. A: Endocytosis. Polymeric, liposomal NPs and small drugs can be loaded to MSC through this route. B: Lipid fusion. MSC surface can be decorated and loaded with drugs through this route, but this is not the only mechanism for liposomal NPs. Figures 2A and B are not drawn to scale. C: Comparison on target tissue accumulation between SPIO-loaded luciferase MSC and unloaded luciferase MSC. MSC were injected into the muscle injury site and localized by 3T external magnetic force. D: Comparison on restenosis efficacy between magnet-guided and non-guided SPIO-loaded MSC in rabbit femoral artery injury model. Tissue

sections were stained with H&E for visualization. E: Enhancement of therapeutic agent accumulation by MSC in a brain glioblastoma model. NP accumulation of NP-loaded MSC was 5-fold higher than NP only in the tumor site (indicated with an arrow). The figure is adapted from [82], [95], and [97].

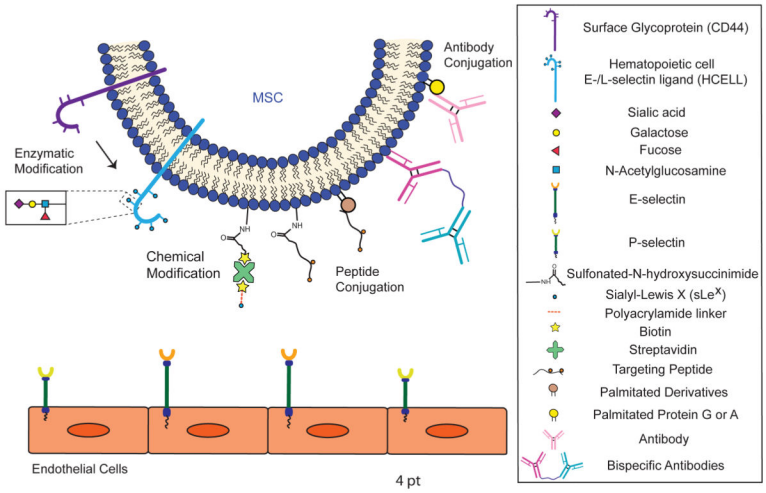

Figure 3.

Schematic diagram of both covalent and non-covalent surface modification techniques used to enhance the homing property of MSC toward the endothelium of therapeutic sites. Four main strategies depicted are: enzymatic modification, chemical modification, peptide conjugation, and antibody conjugation. The sugar chemistry labeling for HCELL (in the left rectangle) is adapted from [150]. Figure not drawn to scale.

Table 1

Summary of the select examples of the applications of MSC engineered with various modalities

2.1.1 Viral transduction	Akt1	Repair heart	Reduced intramyocardial inflammation, regenerated 80–90% lost myocardial volume, and recovered systolic and diastolic cardiac function	[29]
	TGF-β3	Repair cartilage	Significant differentiation towards chondrogenic lineage with deposition of ECM, rich in type II collagen and glycosaminoglycans	[41]
	scFvCD20-TRAIL	Treat non-Hodgkin's lymphoma	Achieved 65% of tumor regression compared to control through intrinsic and extrinsic apoptotic pathway	[60]
2.1.2 Non-viral transfection	Brain-derived neurotropic factor	Treat neurological disease	Displayed that more than 80% of cells expressed BDNF and lasted for 15 days *in vitro*	[26]
	CXCR4 mRNA	Increase homing	Detected mRNA within 30 mins of transfection and persisted in the cytoplasm for about 9 days	[34]
	Anti-mir-138	Regenerate bone	Resulted in the regeneration of bone with good vascularization	[46]
2.2.1 Cellular internalization	PTX	Treat cancer	Internalized PTX into the Golgiderived vesicles; exerted cytotoxic effect on human pancreatic cancer cell line (CFPAC-1) with an IC50 of 10.24 ng/mL	[84]
	SPIO	Regenerate skeletal muscle	Significantly increased the localization of MSC upon exposure to external magnetic force and showed superior muscle regeneration *in vivo*	[95]
	Mesoporous silica NP with Imaging agents (FITC, NIR dye ZW800, Gd^{3+} and ^{64}Cu)	Visualize tumor	Stably integrated to MSC without affecting their intrinsic properties; improved tumor homing by 5.2-fold in the murine glioblastoma model compared with that of NP only	[97]
2.2.2 Lipid fusion	SLe^X	Improve homing to inflamed tissue	Promoted cell rolling and Pselectin interaction by 52 % compared to unmodified MSC at a shear stress of 0.5 dyn/cm^2	[87]
2.3.1 Enzymatic modification (by engineering cell surface glycoproteins using FTVI)	HCELL (active site of HCELL = SLe^X ligand)	Increase homing to bone marrow	Achieved firm adhesion and transmigration of MSC to the microvascular vessel in bone marrow under hemodynamic shear stresses of up to 30 dyn/cm^2	[113]
2.3.2 Chemical modification (by covalently coupling targeting ligands)	SLe^X	Repair inflamed tissue	Promoted cell rolling, adhesion, and migration *in vitro* and *in vivo*; increased 56% of migration efficiency towards inflamed murine ear compared to control	[116], [121]
	Polymer-Ale hybrid	Regenerate bone	Enhanced homing to both human and rat bone fragments without affecting the proliferative and differentiative potential of MSC	[120]

2.3.3 Non-covalent modification (by employing molecular recognition techniques)	**2.3.3.1 Antibody conjugation**	Bispecific antibody	Repair myocardial infarction	Significantly increased the homing of MSC at the target site and reduced the fibrosis density compared with the unmodified MSC	[132]
	2.3.3.2 Peptide conjugation	Palmitated-ischemiahoming peptide	Repair myocardial infarction	Enhanced localization (10-fold increase) of peptide-coated MSC compared to their control *in vivo*	[134], [135]

Table 2

The advantages and disadvantages of each MSC engineering method

2.1.1 Viral transduction	High transduction efficiency	Long-term safety and regulatory issues; size limitation for the insert (<9kb); labor-intensive; expensive; may cause insertion mutagenesis
2.1.2 Non-viral transfection	Potentially safer than viral transduction; flexible insert size; cost-effective; simple and fast; high reproducibility	Low transfection efficiency; transient gene expression
2.2.1 Cellular internalization	Cargo diversity; simple and fast; high loading efficiency	Cargo may affect MSC viability; unable to decorate the cell surface
2.2.2 Lipid fusion	Enables cell surface decoration	Complicated; cargo release profile needs to be optimized
2.3.1 Enzymatic modification	Improve cell homing to affected endothelial beds without compromising MSC's native properties; induce transmigration mediated by selectin; simple	Limited to modifications on the existing endogenous glycoproteins; may loose targeting moiety due to internalization or shedding of the cell over time (Transient expression)
2.3.2 Chemical modification	Improve cell homing property without compromising MSC's native properties; versatile; simple	Transient expression of the targeting moiety
2.3.3.1 Antibody conjugation	Improve cell homing property without compromising MSC's native properties; employs high specific affinity towards a specific antigen; enables multi-targeting; versatile	High production cost on a large scale with high purity; lack of MSC-specific cell markers; Transient expression of the targeting moiety
2.3.3.2 Peptide conjugation	Improve cell homing property without compromising MSC's native properties; versatile; exploits ligandspecific interactions; applicable to a wide range of target tissues and cell types; low production cost on a large scale with high purity; enable to optimize the binding affinity using phage display	Transient expression of the targeting moiety