Growth Factor Gene-Modified Mesenchymal Stem Cells in Tissue Regeneration

This article was published in the following Dove Press journal:
Drug Design, Development and Therapy

Wen-Bo Nie
Dan Zhang
Li-Sheng Wang

Department of Rehabilitation Sciences,
School of Nursing, Jilin University,
Changchun, People's Republic of China

Abstract: There have been marked changes in the field of stem cell therapeutics in recent years, with many clinical trials having been conducted to date in an effort to treat myriad diseases. Mesenchymal stem cells (MSCs) are the cell type most frequently utilized in stem cell therapeutic and tissue regenerative strategies, and have been used with excellent safety to date. Unfortunately, these MSCs have limited ability to engraft and survive, reducing their clinical utility. MSCs are able to secrete growth factors that can support the regeneration of tissues, and engineering MSCs to express such growth factors can improve their survival, proliferation, differentiation, and tissue reconstructing abilities. As such, it is likely that such genetically modified MSCs may represent the next stage of regenerative therapy. Indeed, increasing volumes of preclinical research suggests that such modified MSCs expressing growth factors can effectively treat many forms of tissue damage. In the present review, we survey recent approaches to producing and utilizing growth factor gene-modified MSCs in the context of tissue repair and discuss its prospects for clinical application.

Keywords: growth factor, mesenchymal stem cell, tissue regeneration, genetic engineering

Background

In settings where the human body is unable to partially or fully heal a given tissue injury, the use of stem-cell based regenerative therapies offers great promise as a means of improving patient outcomes.¹ Indeed, such therapies can support heart or kidney transplants, bone reconstruction, or the repair of skin, cartilage, and neurons. In patients suffering from pathological conditions, such therapies can also potentially restore compromised tissue function.²⁻⁴ Mesenchymal stem cells (MSCs) are a form of multipotent stem cell capable of differentiating into a subset of distinct cell types such as myocytes, adipocytes, chondrocytes, and osteoblasts. As they are capable of differentiating into several cell types, homing to target tissues, and secreting growth factors and immunomodulatory compounds, MSCs represent an ideal cell type to use for treating a range of disease types. Importantly, these cells can also be easily obtained and amplified in vitro without engendering substantial ethical concerns, allowing them to be safely and readily used in patients.

Most organs in human adults are limited in their ability to undergo tissue regeneration, instead undergoing scarring that can disrupt organ function. As such, the utilization of MSCs to facilitate true tissue reconstruction rather than scarring represents an ideal means of maintaining normal tissue function in the context of injury. Many studies to date have explored the ability of MSCs to support bone development, restoration of ventricular functional, and improved renal tubular

Correspondence: Li-Sheng Wang
Department of Rehabilitation Sciences,
School of Nursing, Jilin University,
Changchun, People's Republic of China
Tel +86-43185622010
Fax +86-43185619580
Email lishengwang@jlu.edu.cn

function in vivo and in clinical settings.⁴⁻⁶ Unfortunately, however, these cells are limited in their therapeutic efficacy, particularly in contexts where injuries or the associated ischemic damage are severe and irreversible. Indeed, preclinical animal models suggest that MSCs have a poor ability to engraft, and they are also hampered by limited homing and survival in vivo owing to factors including inflammation, ischemia, and anoikis.⁷ One strategy proposed to overcome such limitations centers on the use of MSCs engineered to express specific genes.

Growth factors (GFs) are well known to be key mediators that can support MSC survival and proliferation, in addition to being key drivers of tissue regenerative processes. Many recent studies have utilized MSCs in order to deliver specific GFs to a target site of tissue regeneration either via utilizing cells naturally secreting these factors, or by engineering these cells to overexpress GFs of interest. Indeed, many recent studies have explored the therapeutic potential of MSCs engineered to express particular GFs in a therapeutic context. In the present review, we offer an overview of recent studies exploring the application of GF gene-modified MSCs in the field of tissue repair and reconstruction.

The Relationship Between MSC Biology and GF Secretion

MSCs are a readily isolated cell type that expand rapidly in culture without losing the ability to undergo self-renewal, permitting their use for reconstructing damaged tissues and organs via extensive amplification.⁸ In addition to their multipotent ability to differentiate into a range of cell types, MSCs can orchestrate and enhance proximal or distal cell functionality via paracrine signaling and endocrine mechanisms. Studies have shown MSCs to be capable of promoting tissue regeneration via secreting exosomes and GFs including hepatocyte growth factor (HGF), fibroblast growth factor (FGF), and vascular endothelial growth factor (VEGF).⁹ Additionally, these cells express high levels of factors known to regulate hematopoietic cell function such as CXCL12, vascular cell adhesion molecule 1, interleukin-7, angiopoietin-1 (Ang-1), and osteopontin.¹⁰ Consistent with these findings, in vivo studies also support the fact that the paracrine secretion of GFs by MSCs is a key mechanism whereby they support target tissue healing, as while these cells can migrate to sites of injury, the cells derived therefrom contribute only to a limited degree to therapeutic efficacy. Many recent studies have suggested that the secretion of GFs

and other bioactive molecules may be one of the primary mechanisms whereby MSCs mediate their therapeutic efficacy. These secreted compounds can inhibit a range of processes such as apoptotic cell death and fibrosis,¹¹ in addition to being able to drive angiogenesis,^{12,13} and to regulate the immune response.^{14,15}

Without any exogenous manipulation, MSCs achieve limited therapeutic efficacy due to their poor survival and limited GF secretion upon transplantation. The therapeutic efficacy of MSCs ultimately depend upon the number of cells implanted, the function of these cells, when they are administered, and what condition they are being used to treat.^{9,16-18} Poor MSC engraftment can be attributable to limited cell survival as a consequence of ischemia, anoikis, loss of trophic factors, or localized inflammation.¹⁹ It is thus vital that MSC survival and differentiation be improved following transplantation in order to enhance therapeutic outcomes in treated patients. To that end, studies have explored the use of MSCs modified to express certain exogenous genes that can enhance their ability to promote angiogenesis and target tissue homing.^{13,20} These genetically engineered MSCs can thereby both improve MSC engraftment and functionality, while also allowing for the targeted delivery of therapeutic gene products that can enhance local tissue healing.²¹ Indeed, MSCs can secrete a broad profile of active molecules including hematopoietic growth factors, angiogenic growth factors, trophic molecules, immunomodulatory cytokines, and chemokines. The best-characterized GFs and cytokines produced by these cells are compiled in [Table 1](#). Based on these previous findings, it is clear that engineering MSCs to overexpress GFs may be an optimal means of improving the therapeutic efficacy of these cells.

Vectors Used for GF Overexpression in MSCs

Both non-viral vectors such as lipids or polymers, as well as viral vectors (including retroviruses, adenoviruses, lentiviruses and adeno-associated viruses) have been used to mediate GF overexpression in MSCs. The most common vectors used for such approaches are compiled in [Table 2](#).³¹⁻³⁹ Using viral vectors to insert genes into MSCs is a high transduction efficiency approach that has the potential to induce off-target effects owing to insertional mutagenesis.^{32,35,40,41} Viral systems are also limited by relatively small transgene cargo capacity, high production cost, difficulties in production and scale-up, and adverse

Table 1 Secretome of Mesenchymal Stem Cells

Type of Secreted Factors	Active Molecules	Ref
Hematopoietic growth factors	SCF, FLT3LG, Thrombopoietin, IL-3, IL-6, GM-CSF, M-CSF	[22–24]
Angiogenic growth factors	HGF, VEGF, Angiopoietin, PDGF, IGF-I, FGF-2, FGF-4, FGF-7	[22,23,25,26]
Trophic molecules	Adiponectin, Adrenomedullin, Osteoprotegerin, MMP10, MMP13, TIMP-1, TIMP-2, TIMP-3, TIMP-4, Leptin, IGFBP-1, IGFBP-2, IGFBP-3, IGFBP-4, BDNF, GDNF, NGF, PIGF	[22,23,27]
Immunomodulatory cytokines	IL-1 α , IL-1 β , IL-2, TSG-6, OSM, IL-7, IL-10, IL-11, IL-12, IL-13, IL-16, IFN- γ , TNF- α , LIF, TGF- β , MIF	[23,24,28]
Chemokines	CCL1, CCL2, CCL5, CCL8, CCL11, CCL16, CCL18, CCL22, CCL23, CCL24, CCL26, CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL8, CXCL11, CXCL12, CXCL13, CX3CL1, XCL1	[22,23,29,30]

Abbreviations: SCF, stem cell factor; FLT3LG, Fms-related tyrosine kinase 3 ligand; IL, interleukin; GM-CSF, granulocyte macrophage colony-stimulating factor; M-CSF, macrophage colony-stimulating factor; HGF, hepatocyte growth factor; VEGF, vascular endothelial growth factors; PDGF, platelet-derived growth factor; IGF, insulin-like growth factor; FGF, fibroblast growth factor; MMP, matrix metalloproteinase; TIMP, tissue inhibitor of metalloproteinase; IGFBP, insulin-like growth factor-binding protein; BDNF, brain-derived neurotrophic factor; GDNF, glial cell-derived neurotrophic factor; NGF, nerve growth factor; PIGF, placenta growth factor; TSG, tumor necrosis factor-stimulated gene; OSM, oncostatin; IFN, interferon; TNF, tumor necrosis factor; LIF, leukemia inhibitory factor; TGF, transforming growth factor; MIF, macrophage migration inhibitory factor; CCL, C-C motif chemokine ligand; CXCL, C-X-C motif chemokine ligand; CX3CL, C-X3-C motif chemokine ligand; XCL, X-C motif chemokine ligand.

immune reactions. There are advantages and disadvantages to all known viral vectors, with the selection of an appropriate vector being dependent upon transduction rates and the desired duration of treatment and target gene expression. It is also essential that such modified MSCs be extensively screened for safety reasons, thus potentially reducing the cost-effectiveness of such approaches in a clinical context.

To avoid the limitations of viral vectors, non-viral vectors such as nanoparticles (NPs) or cationic liposomes have been utilized to deliver vectors into MSCs. These alternative delivery strategies are more scalable and flexible, easier to synthesize and target to tissues, less likely to drive immune stimulation, and more amenable to scale-up manufacturing.³⁷ However, the disadvantages of non-viral vectors can include their transient expression with low efficiencies, and their potential for associated toxicity. He et al⁴² utilized the cationic polymer pullulan-spermine to overexpress HGF encoded in the pMEX vector in MSCs, resulting in high in vitro HGF expression.⁴³ In contrast to such success, however, Tan et al⁴⁴ found that such plasmid-containing liposomes were only able to mediate FGF expression in BMSCs at a relatively low transfection efficiency, although they were able to achieve expression at levels sufficient to support periodontal regeneration. Still other authors have utilized lipid-based NPs to achieve target gene expression in MSCs for a sustained period of time.^{45,46} These vectors, however, have recently been

suggested to have the potential to induce genotoxicity, thus potentially mediating oncogenesis.^{47,48} It is thus important to weight the relative costs and benefits of these different strategies to MSC engineering in order to produce a safe, effective, and sustainable approach for clinical use.

The Impact of GF Overexpression on MSCs in Tissue Regeneration

The Primary Impact of GF Gene-Modified MSCs in Tissue Regeneration

Many previous studies have explored the ability of GFs to regulate MSC growth in vitro via adding these GFs to cell culture media and/or by inhibiting their cognate receptors, allowing for the study of concentration-dependent effects. When MSCs are treated with GFs including FGF-2, PDGF-B, TGF- β 1, and VEGF-A, this has been shown to result in enhanced production and secretion of GFs by MSCs.⁴⁹ As such, overexpressing target GFs in MSCs may be able to yield similar therapeutic effects to those observed upon adding recombinant GFs to MSC cultures, although they can also affect the biology of cells in a therapeutically uncertain manner. Indeed, the secretion of exosomes and GFs such as HGF, FGF-B, and VEGF is potentially key to the regenerative abilities of MSCs.²³ When these GFs are overexpressed, this is associated with significant enhancement of MSC-mediated regeneration of tissues, making

Table 2 Summary of Common Vectors Used for GF Expression in MSCs

Types of Vectors	Commonly Used Examples	Transduction Efficiency in MSCs	Advantages	Disadvantages	Preclinical or Clinical Application	Ref
Viral vector	Retrovirus	74.8–85.6%	Long-term stable gene expression	Insertional mutagenesis and activation of oncogenes	Preclinical	[32]
	Adenovirus	76.2–80%	Lower risk of genotoxicity	Transient gene expression	Preclinical	[35]
	Lentivirus	96.3–99.1%	Long-term stable gene expression	Insertional mutagenesis	Clinical	[32]
	Adeno-associated virus	≥ 65%	Long-term gene expression; non-immunogenic	Limited transport capacity	Preclinical	[36]
Nonviral vector	Physical methods					
	Electroporation	68.0–80.0%	Moderate transfection efficiency	Low cell viability	Preclinical	[34,37]
	Nucleofection	51.0–88.0%	Moderate/High transfection efficiency	Low cell viability	Preclinical	[34,38]
	Chemical methods					
	Lipid and polymeric agents	2.0–35.0%	Low immunogenicity	Low transfection levels, cytotoxic	Preclinical	[33,38]
	Dendrimers	10.0–17.0%	Low cytotoxicity and immunogenicity	Low transfection levels	Clinical	[33,39]
	Inorganic nanoparticles	25.0–75.0%	Wider availability, controlled delivery, low toxicity	Only moderate transfection efficiencies	Preclinical	[31,33]

Abbreviations: GF, growth factor; MSC, Mesenchymal stem cell; Ref, references.

such GF overexpression strategies a focus of key therapeutic interest. A general overview of the therapeutic utilization of GF gene-modified MSCs is shown in [Figure 1](#). First, MSCs are extracted from humans or animals, identified, and amplified. Second, the GF gene of interest is integrated into the vector and jointly introduced into the MSCs. Third, GF modified MSCs are delivered to the target tissues of the recipient organism wherein they can play a therapeutic role via secreting GFs, promoting angiogenesis, and enhancing homing functions.

The Selection of GFs in MSC Modification

Initially selection of GFs used to treat MSCs was based on prior understanding of the role of these GFs in cellular differentiation and morphogenesis, with experiments being aimed at exploring the ability of these GFs to drive MSC differentiation towards particular lineages. For example, HGF is a multifunctional factor produced by MSCs

which can bind to its cognate receptor c-Met on cells of the vascular endothelium.⁵⁰ Studies using mice lacking expression of HGF in specific tissues highlighted the ability of this GF to support tissue repair and regeneration,⁵¹ and the implantation of MSCs overexpressing HGF led to enhanced left ventricular remodeling,⁵² reductions in neurological deficits,⁵³ and enhanced liver function.⁴³ Similarly, MSCs engineered to overexpress VEGF have been shown to enhance the viability of cells in the context of in vitro hypoxia and can also improve capillary formation in animal models of myocardial infarction,⁵⁴ hind limb ischemia,⁴⁹ and skin defects.⁵⁵ Certain GFs exhibit similar repair effects in MSCs for many tissue types. For instance, angiopoietin-1 (Ang-1) is a growth factor that specifically acts on endothelial cells and can drive angiogenesis.⁵⁶ MSCs overexpressing Ang-1 have been shown to inhibit cardiac remodeling and to drive improved myocardial angiogenesis and arteriogenesis relative to

Figure 1 An overview of the therapeutic utilization of GF gene-modified MSCs.
Abbreviation: GF, growth factor; MSC, Mesenchymal stem cell.

control MSCs.⁵⁷ Such cells were also able to markedly reduce pulmonary inflammation⁵⁸ and to facilitate tissue repair.⁵⁹ MSCs overexpressing Ang-1 have also been shown to improve wound healing in a rat model system, enhancing angiogenesis in addition to dermal and epidermal tissue regeneration.⁶⁰ Tissue-specific repair factor modifications enhance the repair capabilities of MSCs in specific tissues. For example, BDNF promotes the survival and differentiation of neuronal tissue by acting on receptor kinases,⁶¹ and BDNF-MSCs have primarily been used to promote the survival of neurons in the context of brain injury.^{62,63} Similarly, TGF family proteins are closely linked to MSC survival and differentiation.^{64,65} In particular, TGF- β superfamily genes are often used to drive MSC chondrogenic differentiation.⁶⁶ Therefore, TGF- β 1 has been chosen to engineer rat MSCs to support enhanced regeneration of cartilage.⁴² Hence, the selection of a particular GF for use in the modification of MSCs depends upon the effect of growth factors on MSCs and also on the response of the damaged tissue itself.

MSCs Overexpressing Multiple GF Genes Exhibit Therapeutic Utility

The primary mechanism whereby such gene-modified MSCs contribute to tissue repair is via the secretion of these multifactorial GFs rather than via their ability to differentiate into particular cell types, with these cells serving key roles in inhibiting fibrosis and inflammation while promoting angiogenesis.⁴⁹ Some studies have modified MSCs to express multiple synergistic genes in an effort to enhance their therapeutic utility. For example, IGF-1 is a GF that promotes cell survival,⁶⁷ whereas HGF promotes angiogenesis while suppressing fibrosis and inflammation.¹⁸ In a rat model of myocardial infarction, human adipose-derived stem cells that continuously produced IGF-1 and HGF were able to achieve a 1.3-fold increase in medium-sized blood vessel density at the infarct border zone relative to control cells.⁶⁸ In another study using a porcine model of myocardial infarction, such MSCs overexpressing HGF and IGF-1 were able to drive angiogenesis and suppress

inflammation more effectively than other cells, although these cells also exhibited enhanced fibrosis suggesting that combined IGF-1 and HGF exposure over extended periods of time can induce both beneficial and counterproductive effects.⁶⁹ This suggests that the preparation of MSCs secreting both IGF-1 and HGF may not be an effective means of synergistically effective cardiac repair, with the elevated levels of either factor in the local environment potentially contributing to this effect.

The Role of Exosomes Derived from GF-Modified MSCs in Tissue Regeneration

Multiple studies have indicated a role for MSCs in regenerative medicine through their paracrine effects and ability to produce exosomes.^{17,70} Encapsulated with a lipid bilayer, exosomes can protect their contents from degradation and can transport a variety of small biomolecules including mRNAs, miRNAs, and proteins to surrounding cells. Moreover, MSC sources and culture conditions have been shown to influence the regenerative responses induced by exosomes, as a number of GFs can be detected in MSC-derived exosomes, including HGF, IGF family members, FGF2, and platelet-derived growth factor-AA (PDGF-AA).⁶⁹ As natural vesicles suitable for gene delivery, MSC-derived exosomes exhibit a broad range of therapeutic effects, and can mediate tissue repair, immunological regulation, and inflammatory control.^{70,72} Moreover, recent studies have revealed that MSC-derived exosomes can mediate therapeutic benefits in animal disease models, with previous studies of bone fracture, cutaneous wound, myocardial infarction, and acute hepatic injury all having demonstrated the clinical utility of such exosomes.^{16,17,70,73} Exosomes can modulate the differentiation and migration of MSCs in a targeted manner, offering an opportunity to promote tissue regeneration in a cell-free manner. Genetic manipulation can also be used to control the levels of such GFs in these exosomes, as in studies in which huc-MSCs were engineered to secrete GFs in a controlled fashion over an extended period.⁷¹ Such genetically modified MSC-derived exosomes may thereby be able to mediate tissue regenerative benefits, making them ideal for future therapeutic regenerative regimens.

Preclinical Use of GF-Modified MSCs in Tissue Regeneration

The therapeutic value of MSCs stems largely from their ability to mediate angiogenesis and tissue regeneration,⁷⁵ secreting GFs and exosomes to achieve therapeutic efficacy

and homing to target tissue sites.^{9,16,17} A number of preclinical studies to date have sought to use genetically-modified MSCs that secrete GFs in order to treat a wide range of conditions associated with tissue injuries. A detailed overview of the uses of GF-modified MSCs in preclinical tissue repair studies is given in Table 3.

Central Nervous System (CNS) Lesions

Occlusive cerebrovascular diseases can result in cerebral ischemia and significant neuropathology, leading to the exploration of many modes of treating such diseases including the application of MSC-based therapies. One of the keys to treating CNS lesions is to maintain the integrity of the blood-brain barrier and to reduce edema in the context of ischemia, thus reducing the severity of injury. Importantly, MSCs can home to the CNS *in vivo*, allowing them to improve functional recovery following stroke owing to their ability to drive angiogenesis and neurogenesis while suppressing local inflammation via GF and chemokine secretion.⁸⁸ MSCs overexpressing specific GFs can also help to facilitate efficient CNS tissue regeneration. For example, MSCs overexpressing HGF have shown superior efficacy in reducing neurological deficits in the rat middle cerebral artery occlusion (MCAO) model relative to unmodified MSCs.⁵³ Su et al⁸⁹ found that BMSCs engineered to overexpress GDNF using a lentivirus were able to protect against injury in PC12 cells, highlighting their potential therapeutic value in the context of Parkinson's disease. However, there are still many obstacles to the widespread use of this technique in CNS lesions. Intracerebral injection remains particularly difficult if the lesions are widespread and numerous. In addition, intra-arterial injection will increase risk of embolic events,⁹⁰ and intravenous injections typically result in few cells reaching the target sites.^{91,92}

Ischemic Heart Disease (IHD)

In many nations, the primary cause of morbidity and mortality is myocardial infarction (MI),⁹³ and as such it is one of the most common targets of therapeutic efforts to engineer MSCs to facilitate tissue repair. Indeed, a number of genes have been proposed as targets for MSC-mediated delivery in the context of MI including HO-1,⁹⁴ IGF-1,⁶⁹ Ang-1,^{57,81} SVV,⁹⁵ Bcl-2⁹⁶ and Akt1.^{97,98} Over 30 clinical studies to date have been registered using MSCs for the treatment of MI, but these studies have suggested the need for improved therapeutic efficacy of these MSCs. Angiogenesis mediates clinical benefits via the formation, remodeling, and maturation of blood vessels in injured tissues, making GF engineering an ideal

Table 3 Preclinical Studies of the Use of Genetically Engineered MSCs in Tissue Repair

Disease	Therapeutic Modification	Vector	Cell Type	Cell Counts/ per Animal	Method of Administration	Effects	Ref
Transient MCAO	FGF-2	Replication-incompetent HSV-1 vector	Rat BMMSC	1×10^6 /Rats	Administered intracerebrally	Enhanced survival, reduced infarction volume, improve functional recovery	[76]
Transient MCAO	BDNF	Adenoviral vector	Human MSC	5×10^5 /Rat	Intracerebrally injection	Promotes the survival and differentiation, reduced infarct size	[63]
Transient MCAO	HGF	Multimutated herpes simplex virus type-1 vector	Rat BMMSC	1×10^6 /Rat	Injected into the right striatum	Improved neurological deficits, reduced infarction volume	[53]
Huntington's disease	BDNF	Lentiviral vector	Human BMMSC	5×10^5 cells per hemisphere/ Mouse	Injected bilaterally into the striata with vehicle	Decreased striatal atrophy, reduced anxiety, induced increase in neurogenesis-like activity.	[77]
Parkinson's disease	BDNF	Electroporation	Rat BMMSC	5×10^6 /Rat	Lateral ventricular injection	Reduce the DA metabolic rate, improve the level of DA, and improve the behavior of PD rats	[78]
Traumatic Brain Injury	BDNF	Adenoviral vector	Rat BMMSC	1×10^6 /Rat	Intraventricular injection	Increased BDNF levels, attenuated neuronal injury	[62]
Myocardial infarction	VEGF	Bile acid-modified polyethyleneimine	Human BMSC	1×10^6 /Rat	Injected intramyocardially into the contracting wall bordering the infarct	Improved cell viability, enhanced capillary formation in the infarcted region, attenuated left ventricular remodeling	[54]
Myocardial infarction	HGF	Retroviral vector	Rat BMMSC	2×10^6 /Rat	Injected into three points around the infarct area	Improved left ventricular function, decreased infarcted scar area, and increased angiogenesis.	[52]
Acute myocardial infarction	HGF and IGF-I	Lentiviral vectors	Pig MSC from adipose tissue (paMSC)	50×10^6 /Pig	7–8 injections surrounding the infarcted area	Reduced inflammation, promoted angiogenic processes	[69]
Myocardial fibrosis	HGF	Lentiviral vector	Rat BMMSC	2×10^7 /Rat	Injected into the border zone of infarcted heart tissue	Enhance cell survival, improve cardiac function, stimulate angiogenesis, and reduce myocardial fibrosis	[79]
Myocardial infarction	VEGF	Adenovirus vector	Rat BMMSC	5×10^6 /Rat	Injected into the border zone surrounding the infarcted area	Induced myocardial angiogenesis and cardiomyocyte regeneration, prevented progressive scar formation and heart dysfunction	[80]

(Continued)

Table 3 (Continued).

Disease	Therapeutic Modification	Vector	Cell Type	Cell Counts/ per Animal	Method of Administration	Effects	Ref
Acute myocardial infarction	Ang-I	Recombinant adenoviruses	Rat BMMSC	5×10^6 /Rat	Injected into the border zone surrounding the infarct anteriorly and laterally	Increased capillary density and reduced infarct size	[57]
Ischemic myocardium	Angiopoietin	Adenovirus vector	Rat BMMSC	3×10^6 /Rat	Injected into the ischemic myocardium	Induced differentiation, promoted angiogenesis, improved cardiac function	[81]
Myocardial infarction	Survivin	Lentiviral vector	Rat BMMSC	2×10^6 /Rat	Intra-myocardial injections	Increased capillary density reduced the infarct size, inhibited collagen deposition, and further improved cardiac function	[82]
Radiation-induced intestinal injury	HGF	Adenoviral vectors	Human UC-MSCs	2×10^6 /Mouse	Injected intravenously via the tail vein	Improved intestinal histopathology, reduced inflammation, and increased the proliferation and decreased the apoptosis of intestinal epithelial cells.	[83]
Radiation-wound injury	PDGF-A and BD-2	Adenoviral vector	Rat BMMSC	1×10^7 /Rat	Injected into the wound bed and margin of the excisional wound.	Resulted in better granulation formation/maturation and skin appendage, promoted wound healing regeneration	[84]
Radiation-induced lung injury	TGF- β type II receptor	Adenoviral vector	Mouse BMMSC	5×10^5 /Mouse	Intravenously injected	Attenuated early lung injury and improved survival and lung fibrosis	[85]
Radiation-induced liver injury	NGF	Plasmid vector	Mouse BMMSC	5×10^{10} /Mouse	Intravenously injected	Inhibited the apoptosis of mouse hepatic cells induced by radiation, improved the survival rate of mice	[86]
Cartilage defects	TGF- β I	Pullulan-spermine (nonviral gene vector)	Rat BMMSC three-dimensional (3D) reverse transfection system	1×10^6 cells per scaffold/Rat	TGF- β I gene-transfected MSC seeded gelatin sponge was implanted to the full-thickness cartilage defect	Promoted chondrogenesis of MSCs, and improved cartilage regeneration.	[42]
Osteopenia	BMP-2	Adeno-associated virus	Mouse BMMSC	1×10^6 /Mouse	Intravenously injected	Increased bone mineral density and bone mineral content, promoted proliferative capabilities of cells	[87]
Limb ischemia	VEGF	Lentiviral vectors	Human BMSC	1×10^6 /Mouse	Injected into the tail vein	Induced the migration of endothelial cells and enhanced blood flow restoration	[49]

(Continued)

Table 3 (Continued).

Disease	Therapeutic Modification	Vector	Cell Type	Cell Counts/ per Animal	Method of Administration	Effects	Ref
Skin defect wound	VEGF	Recombinant adenovirus	Human UC-MSC	—	Injection into the wound	Promotes vascular endothelial cell division, proliferation and induction of vascularization	[55]
Chronic skin wound	Ang-I	Recombinant adenovirus vector	Rat BMMSC	1×10^6 /Rat	Injected intradermally at the margin of the excisional wound at four injection sites	Promoted wound healing with increased epidermal and dermal regeneration, and enhanced angiogenesis	[60]
Periodontal regeneration	FGF-2	Plasmid - liposome complexes	Dog BMMSC	1×10^7 /Dog	Transplanted into root furcation defects	Accelerated the proliferation, induced morphogenesis	[44]
Liver fibrosis	HGF	Plasmid vector	Human UCB-MSCs	1×10^6 /Rat	Injected via the caudal vein	Migrated into the injured liver and expressed hHGF; improved the liver morphology and reversed liver damage	[43]
Lung injury.	Ang-I	Lentiviral vector	Mouse BMMSC	-	Intravenously administered via the jugular vein	Attenuated the inflammatory reaction and vascular leakage, improved lung histopathological changes	[59]
Severe acute lung injury	Ang-I	Nuclear-targeting electroporation	Murine BMMSC	2.5×10^5 /Mouse	Infused via a jugular venous canula	Reduced pulmonary inflammation, improved both alveolar inflammation and permeability	[58]
Acute kidney injury	HGF	Adenovirus vector	Human UC-MSC	1×10^6 /Rat	Injected via the left carotid artery	Promoted proliferation, decreased apoptosis and inflammation, and promoted the amelioration of renal function	[50]

Abbreviation: MSC, Mesenchymal stem cell; MCAO, middle cerebral artery occlusion; FGF, fibroblast growth factor; HSV, herpes simplex virus; BMMSC, bone marrow-derived mesenchymal stem cell; BDNF, brain-derived neurotrophic factor; HGF, hepatocyte growth factor; DA, dopamine; PD, Parkinson's disease; VEGF, vascular endothelial growth factors; IGF, insulin-like growth factor; Ang, angiotensin; PDGF, platelet-derived growth factor; BD, beta-defensin; TGF- β , transforming growth factor-beta; NGF, nerve growth factor; BMP, bone morphogenetic protein; UC-MSC, umbilical cord-derived mesenchymal stromal cell; UCB-MSC, umbilical cord blood-derived mesenchymal stem cell.

means of achieved such angiogenic efficacy in a therapeutic setting. Moreover, among angiogenic growth factors, the HGF/Met pathway is a key mediator of cardiovascular remodeling following tissue injury,⁹⁹ with HGF mediating the migration and expression of cardiac-specific markers in MSCs.¹⁰⁰ Many studies have utilized murine, rat, and porcine models of MI to confirm the ability of such HGF-expressing MSCs to enhance cardiac function, drive angiogenesis, and decrease myocardial fibrosis.^{79,101-103} In addition, human BMSCs expressing HGF have been shown to have enhanced anti-arrhythmic properties.¹⁰⁴ Following the delivery of these modified cells to the infarcted region, low local nutrient and oxygen levels can result in poor survival and engraftment efficiency. VEGF is known to enhance the survival of these and other cell types

upon transplantation in damaged tissues.¹⁰⁵ Normally, angiogenesis in the infarcted tissue is not sufficient to meet the needs of the remaining viable myocardial tissue, thereby compromising contractile compensation.⁸⁰ Moon et al⁵⁴ found that MSCs overexpressing VEGF were able to induce a 1.4-fold increase in VEGF expression upon hypoxic exposure relative to cells grown under normoxic conditions, and these modified MSCs were able to facilitate the enhanced microvascularization of infarcted myocardial tissues.

Musculoskeletal Defects and Skin Injuries

Bone, muscle, and skin are all highly metabolized tissues with a relatively high vascular supply, based on the homeostasis of biomaterial structures that need to be studied for

growth and remodeling.¹⁰⁶ Kumar et al⁸⁷ found that mice transplanted with MSCs engineered to overexpress bone morphogenetic protein 2 (BMP2) exhibited increased bone mineral density and content and improved BMSC proliferation relative to control animals, with a corresponding improvement in bone formation. Dental pulp stem cells overexpressing HGF have also been shown to prevent bone loss in the early phase of ovariectomy-induced osteoporosis.¹⁰⁷ MSCs engineered to overexpress Ang-1 are also able to facilitate wound healing as well as dermal and epidermal regeneration and angiogenesis.⁶⁰ In addition, tissue engineering is usually achieved via inserting stem cells into three-dimensional scaffolds that are induced to generate new cells.^{6,108} GF-modified MSCs have been widely used in this innovative treatment for musculoskeletal defects and skin wounds, with many studies having explored optimal tissue engineering approaches to improving the efficiency of cells, scaffolds, and bioactive factors.³³ The most commonly studied technique is to add supplemental growth factors that locally provide signals that mimic the process of bone regeneration.¹⁰⁹ It is therefore important to design systems that provide this biological cue in a time-controlled manner so as to mimic the normal bone healing process. Brunger et al attempted to develop a system using poly-L-lysine to immobilize a lentivirus encoding TGF- β 3 in a 3D woven poly scaffold to induce robust and sustained cartilaginous extracellular matrix formation by hMSCs.¹¹⁰

Radiation Injury

Certain tissues including the lungs, intestines, and bone marrow are highly radiation sensitive. While hematopoietic stem cells can regenerate the bone marrow, strategies to mediate similar regeneration of lung and intestinal tissue are limited. GF-overexpressing MSCs may therefore represent an ideal approach to regenerating tissues following radiation injury and associated damage. For example, in a model of radiation-induced lung fibrosis, MSCs overexpressing HGF were shown to home to damaged lung tissue wherein they could promote epithelial cell proliferation and survival, thereby decreasing local inflammation and fibrosis.¹⁰⁴ Similarly, MSCs engineered to overexpress TGF- β 2 using an adenoviral vector were able to reduce lung injury and protect alveolar type II cells from radiation-induced apoptosis and DNA damage while reducing local inflammation, highlighting the benefits of GF production by MSCs in a paracrine manner.⁸⁵ BMSCs engineered to express VEGF were similarly able to improve radiation-induced tissue injury repair owing to their ability to drive angiogenesis and regeneration of muscle fibers.¹¹¹

BMSCs modified to express both BD2 and PDGF-A using an adenoviral vector were also able to improve wound healing in a model of radiation-induced wounding.⁸⁴ MSCs overexpressing HGF suppress local inflammation and enhance small intestinal recovery in a murine model of radiation induced intestinal injury.⁸³ Irradiation of cardiac tissue can result in late cardiovascular complications, and HGF can reduce such radiation-induced cardiac injury in a model of irradiation-induced heart disease.¹¹² Adenoviral-mediated overexpression of HGF can also prevent radiation-induced hematopoietic damage¹¹³ and can reduce radiation induced hepatic damage in a rat model system.¹¹⁴

Other Tissue Injuries and Diseases

In addition to the diseases mentioned above, MSCs modified to overexpress GFs have been employed to treat a wide range of tissue injuries and diseases in preclinical studies. Studies have shown that MSCs overexpressing HGF and Ang-1, respectively, can improve therapeutic outcomes in ischemia/reperfusion injury in the lung¹¹⁵ and in a Phosgene-induced model of lung injury owing to their ability to decrease pulmonary inflammation and endothelial permeability.¹¹⁶ Furthermore, MSCs modified to overexpress HGF have been shown to improve such AKI in a rat model of ischemia/reperfusion injury via reducing kidney inflammation and apoptotic cell death, thus making these cells of value to human therapeutic implementation.⁵⁰ Moreover, MSCs expressing HGF can also enhance liver regeneration, making them viable for the treatment of those patients suffering from liver fibrosis or cirrhosis.⁴³

Clinical Trials Utilizing Genetically Modified MSCs

Given the number of preclinical studies demonstrating the potential utility of genetically modified MSCs, it is perhaps unsurprising that a number of clinical trials have been or are currently being conducted exploring the clinical value of such therapeutic approaches. To date over one thousand MSC-based trials have been conducted globally as reported in the US National Institute of Health database (ClinicalTrial.gov) in order to evaluate the safety and efficacy of either autologous or allogeneic MSCs. These trials are primarily focused on treating human diseases such as cancer,¹¹⁷ metabolic and inflammatory diseases such as chronic obstructive pulmonary disease,¹¹⁸ or adult respiratory distress syndrome.¹¹⁹ These studies are primarily reliant upon the use of unmodified MSCs for

these clinical efforts, with very few studies to date utilizing genetically modified MSCs. In 2006, Ripa et al published the results of a trial initiated in 2003 piloting the combination of VEGF gene therapy and stem cell mobilization in patients with severe chronic ischemic heart disease, finding this approach to be safe in humans¹²⁰ (NCT00135850). Another relevant study aims to explore the use of MSCs overexpressing BDNF for the treatment of Huntington's disease (HD) patients in a pre-cellular therapy observational study¹²¹ (NCT01937923). At present, however, this study has only enrolled a cohort of individuals early-stage HD in order to characterize their clinical and biomarker findings at baseline for comparisons to a planned future Phase 1 trial safety and tolerability trial applying these BDNF-modified MSCs. This trial has been submitted as an Investigational New Drug application to the Food and Drug Administration.¹²²

A number of challenges still face the clinical implementation of GF gene-modified MSC-based therapies. Of particular difficulty is the production of clinical grade therapeutic products, as such cellular and gene therapies differ from traditional pharmaceutical compounds, instead representing a form of heterogenous biological product that can vary in response to a wide range of culture conditions. Modified MSCs also have the potential to become malignant upon transplant, and the use of recombinant viral vectors to manipulate these cells poses a significant safety concern.¹⁰⁹ Minimizing variability in sample preparation while still remaining cost-effective thus represents a significant challenge. Therefore, the production of modified MSCs for clinical applications must comply with the Good Manufacturing Practice (GMP) standards for medicinal products. These recommended approaches include product safety, cell characterization, and manufacturing process control.¹²³ Cell donors must be screened carefully and the stem cells expanded in the GMP production facilities should be tested using standardized procedures to assess their viability, sterility, genetic stability, tumorigenicity, adventitious agents, pyrogenicity, and mycoplasma infection status. Modified MSCs also have to be verified with respect to their identities, purity, stability and potency. In addition, the biological activity and toxicity of stem cell products must be tested in an applicable animal model under Good Laboratory Practice (GLP) conditions prior to administration into humans.¹²⁴ To ensure product efficacy, however, it is essential that these standardized production procedures do not compromise therapeutic efficacy. The fate of modified MSCs upon intravenous injection is also uncertain, as previous trials of unmodified MSCs have achieved limited efficacy owing to

their quick elimination from circulation.¹²⁵ Therefore, to achieve significant functional benefits, this strategy requires a defined selection of the number and type of stem cells to be delivered, an explicit vector application method, and fixed transduction efficiency and time of administration. In addition, the design of novel bioactive materials such as three-dimensional spheroids¹²⁶ and nano-active scaffolds¹⁰⁹ to bolster stem cell survival, signaling, and function at the target site can also help to increase the cost-effectiveness of the applications of modified MSCs for tissue repair.

At present it remains unclear as to whether it will be legally permissible to utilize genetically modified MSCs for clinical treatment. The potential consequences of utilizing such cells in humans are not well understood, and as such the safety of these approaches needs to be more thoroughly examined in animal model systems in order to identify means overcoming any potential safety issues. In addition, many of the ethical issues associated with genetically-modified MSC research are similar to those arising in other MSC-based interventions. Efforts to address these issues typically focus upon minimizing the risk of harm, emphasizing the importance of informed consent and information disclosure, reducing the potential for overpromising, limiting excessive expectations and therapeutic misconceptions, and avoiding pressure from commercial entities and disease constituencies to move quickly into the clinic.^{125,127} In addition, justice is a necessary consideration given that stem cell interventions can be extraordinary costly and labor-intensive,¹²³ as can many other novel biotechnologies. Justice necessitates that additional attention be paid to the cost of genetically modified MSC interventions in an effort to make them available, effective, and safe, with the goal of reducing unfair disparities in treatment accessibility. These ethical considerations continue to provide crucial guidance for the clinical application of these approaches not only for the trials specifically considered, but also for investigators exploring new translational medicine pathways.

Current Challenges and Future Prospects

The therapeutic utility of GF gene-modified MSCs has been a focus of increasing research interest in recent years owing to their enhanced ability to suppress inflammation, home to target tissues, regulate immune responses, and facilitate tissue repair. Several preclinical and clinical

studies have utilized MSC-based therapeutic strategies for treating a range of disorders and injuries. While efforts to modify MSCs to overexpress defined GFs are still in their early stages and are far from clinical application, although they offer a potentially ideal means of directed tissue regeneration. MSCs alone are limited in their ability to home to and survive in injured tissues, making the modification of MSCs to express such GF genes vital in order to facilitate more robust regenerative medicine approaches. While the outcomes of many of the studies reported in this review are promising, there remain many challenges which must be overcome. These include the need to optimize delivery strategies in human patients while simultaneously preventing immunogenicity or tumor formation. Preclinical findings highlight the safety and therapeutic efficacy of these GF-modified MSCs for the treatment of tissue damage.

In addition, large-scale, multi-center clinical trials are needed to conclusively demonstrate the long-term beneficial effects of such therapies.

Further ongoing clinical studies and efforts to demonstrate the long-term beneficial effects will help to ensure that these promising therapeutic tools soon become available to patients as a novel and efficacious form of regenerative medicine.

Acknowledgments

The authors would like to thank the Jilin Scientific and Technological Development Program and the Interdisciplinary Research Funding Program for PhD Students of Jilin University, for their support in developing this paper.

Data Sharing Statement

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

Funding

This study was supported by Jilin Scientific and Technological Development Program [grant number 20190304041YY] and the Interdisciplinary Research Funding Program for PhD Students of Jilin University [grant number 10183201849].

Disclosure

The authors report no conflicts of interest in this work.

References

1. Rajabzadeh N, Fathi E, Farahzadi R. Stem cell-based regenerative medicine. *Stem Cell Investig*. 2019;6:19. doi:10.21037/sci
2. Corey S, Bonsack B, Borlongan CV. Stem cell-based regenerative medicine for neurological disorders: a special tribute to Dr. Teng Ma. *Brain Circ*. 2019;5(3):97–100. doi:10.4103/bc.bc_39_19
3. Lee WS, Kim HJ, Kim KI, Kim GB, Jin W. Intra-articular injection of autologous adipose tissue-derived mesenchymal stem cells for the treatment of knee osteoarthritis: a phase IIb, randomized, placebo-controlled clinical trial. *Stem Cells Transl Med*. 2019;8(6):504–511. doi:10.1002/sctm.18-0122
4. Khan S, Mafi P, Mafi R, Khan W. A systematic review of mesenchymal stem cells in spinal cord injury, intervertebral disc repair and spinal fusion. *Curr Stem Cell Res Ther*. 2018;13(4):316–323. doi:10.2174/1574888X11666170907120030
5. Jayaram P, Ikpeama U, Rothenberg JB, Malanga GA. Bone marrow-derived and adipose-derived mesenchymal stem cell therapy in primary knee osteoarthritis: a narrative review. *PM R*. 2019;11(2):177–191. doi:10.1016/j.pmrj.2018.06.019
6. Qazi TH, Duda GN, Ort MJ, Perka C, Geissler S, Winkler T. Cell therapy to improve regeneration of skeletal muscle injuries. *J Cachexia Sarcopenia Muscle*. 2019;10(3):501–516. doi:10.1002/jcsm.12416
7. Lemcke H, Voronina N, Steinhoff G, David R. Recent progress in stem cell modification for cardiac regeneration. *Stem Cells Int*. 2018;2018:1909346.
8. Grimm D, Egli M, Kruger M, et al. Tissue engineering under microgravity conditions-use of stem cells and specialized cells. *Stem Cells Dev*. 2018;27(12):787–804.
9. Madrigal M, Rao KS, Riordan NH. A review of therapeutic effects of mesenchymal stem cell secretions and induction of secretory modification by different culture methods. *J Transl Med*. 2014;12:260.
10. Mendez-Ferrer S, Michurina TV, Ferraro F, et al. Mesenchymal and haematopoietic stem cells form a unique bone marrow niche. *Nature*. 2010;466(7308):829–834. doi:10.1038/nature09262
11. Rodrigues M, Griffith LG, Wells A. Growth factor regulation of proliferation and survival of multipotential stromal cells. *Stem Cell Res Ther*. 2010;1(4):32. doi:10.1186/sct32
12. Hu MS, Borrelli MR, Lorenz HP, Longaker MT, Wan DC. Mesenchymal stromal cells and cutaneous wound healing: a comprehensive review of the background, role, and therapeutic potential. *Stem Cells Int*. 2018;2018:6901983. doi:10.1155/2018/6901983
13. Shafei AE, Ali MA, Ghanem HG, et al. Mesenchymal stem cell therapy: a promising cell-based therapy for treatment of myocardial infarction. *J Gene Med*. 2017;19:12. doi:10.1002/jgm.2995
14. Liu F, Qiu H, Xue M, et al. MSC-secreted TGF-beta regulates lipopolysaccharide-stimulated macrophage M2-like polarization via the Akt/FoxO1 pathway. *Stem Cell Res Ther*. 2019;10(1):345. doi:10.1186/s13287-019-1447-y
15. Wu R, Liu C, Deng X, Chen L, Hao S, Ma L. Enhanced alleviation of aGVHD by TGF-beta1-modified mesenchymal stem cells in mice through shifting MPhi into M2 phenotype and promoting the differentiation of Treg cells. *J Cell Mol Med*. 2019.
16. Zhang B, Wang M, Gong A, et al. HucMSC-exosome mediated Wnt4 signaling is required for cutaneous wound healing. *Stem Cells*. 2015;33(7):2158–2168. doi:10.1002/stem.1771
17. Furuta T, Miyaki S, Ishitobi H, et al. Mesenchymal stem cell-derived exosomes promote fracture healing in a mouse model. *Stem Cells Transl Med*. 2016;5(12):1620–1630. doi:10.5966/sctm.2015-0285
18. Wang LS, Wang H, Zhang QL, Yang ZJ, Kong FX, Wu CT. Hepatocyte growth factor gene therapy for ischemic diseases. *Hum Gene Ther*. 2018;29(4):413–423. doi:10.1089/hum.2017.217

19. Song H, Song BW, Cha MJ, Choi IG, Hwang KC. Modification of mesenchymal stem cells for cardiac regeneration. *Expert Opin Biol Ther.* 2010;10(3):309–319. doi:10.1517/14712590903455997
20. Li X, Wang Q, Ding L, et al. Intercellular adhesion molecule-1 enhances the therapeutic effects of MSCs in a dextran sulfate sodium-induced colitis models by promoting MSCs homing to murine colons and spleens. *Stem Cell Res Ther.* 2019;10(1):267. doi:10.1186/s13287-019-1384-9
21. Li L, Zhang D, Li P, Damaser M, Zhang Y. Virus integration and genome influence in approaches to stem cell based therapy for andro-urology. *Adv Drug Deliv Rev.* 2015;82-83:12–21. doi:10.1016/j.addr.2014.10.012
22. Sze SK, de Kleijn DP, Lai RC, et al. Elucidating the secretion proteome of human embryonic stem cell-derived mesenchymal stem cells. *Mol Cell Proteomics.* 2007;6(10):1680–1689. doi:10.1074/mcp.M600393-MCP200
23. Tsuji K, Kitamura S, Wada J. Secretomes from mesenchymal stem cells against acute kidney injury: possible heterogeneity. *Stem Cells Int.* 2018;2018:8693137. doi:10.1155/2018/8693137
24. Liu CH, Hwang SM. Cytokine interactions in mesenchymal stem cells from cord blood. *Cytokine.* 2005;32(6):270–279. doi:10.1016/j.cyto.2005.11.003
25. Hoch AI, Binder BY, Genetos DC, Leach JK. Differentiation-dependent secretion of proangiogenic factors by mesenchymal stem cells. *PLoS One.* 2012;7(4):e35579. doi:10.1371/journal.pone.0035579
26. Oskowitz A, McFerrin H, Gutschow M, Carter ML, Pochampally R. Serum-deprived human multipotent mesenchymal stromal cells (MSCs) are highly angiogenic. *Stem Cell Res.* 2011;6(3):215–225. doi:10.1016/j.scr.2011.01.004
27. Caplan AI, Dennis JE. Mesenchymal stem cells as trophic mediators. *J Cell Biochem.* 2006;98(5):1076–1084. doi:10.1002/(ISSN)1097-4644
28. Aggarwal S, Pittenger MF. Human mesenchymal stem cells modulate allogeneic immune cell responses. *Blood.* 2005;105(4):1815–1822. doi:10.1182/blood-2004-04-1559
29. Zhang H, Yang R, Wang Z, Lin G, Lue TF, Lin CS. Adipose tissue-derived stem cells secrete CXCL5 cytokine with neurotrophic effects on cavernous nerve regeneration. *J Sex Med.* 2011;8(2):437–446. doi:10.1111/j.1743-6109.2010.02128.x
30. Arthur A, Cakouros D, Cooper L, et al. Twist-1 enhances bone marrow mesenchymal stromal cell support of hematopoiesis by modulating CXCL12 expression. *Stem Cells.* 2016;34(2):504–509. doi:10.1002/stem.2265
31. Chen X, Nomani A, Patel N, Nouri FS, Hatefi A. Bioengineering a non-genotoxic vector for genetic modification of mesenchymal stem cells. *Biomaterials.* 2018;152:1–14. doi:10.1016/j.biomaterials.2017.10.028
32. Chan J, O'Donoghue K, De L, et al. Human fetal mesenchymal stem cells as vehicles for gene delivery. *Stem Cells.* 2010;23(1):93–102. doi:10.1634/stemcells.2004-0138
33. Park JS, Suryaprakash S, Lao YH, Leong KW. Engineering mesenchymal stem cells for regenerative medicine and drug delivery. *Methods.* 2015;84:3–16. doi:10.1016/j.ymeth.2015.03.002
34. Aslan H, Zilberman Y, Arbeli V, et al. Nucleofection-based *ex vivo* nonviral gene delivery to human stem cells as a platform for tissue regeneration. *Tissue Eng.* 2006;12(4):877–889. doi:10.1089/ten.2006.12.877
35. Santiago-Torres JE, Lovasz R, Bertone AL. Fetal vs adult mesenchymal stem cells achieve greater gene expression, but less osteoinduction. *World J Stem Cells.* 2015;7(1):223–234. doi:10.4252/wjsc.v7.i1.223
36. Stender S, Murphy M, O'Brien T, et al. Adeno-associated viral vector transduction of human mesenchymal stem cells. *Eur Cell Mater.* 2007;13:93–99; discussion 99. doi:10.22203/eCM.v013a10
37. Hamann A, Nguyen A, Pannier AK. Nucleic acid delivery to mesenchymal stem cells: a review of nonviral methods and applications. *J Biol Eng.* 2019;13(1):7. doi:10.1186/s13036-019-0140-0
38. Santos JL, Pandita D, Rodrigues J, Pego AP, Granja PL, Tomas H. Non-viral gene delivery to mesenchymal stem cells: methods, strategies and application in bone tissue engineering and regeneration. *Curr Gene Ther.* 2011;11(1):46–57. doi:10.2174/156652311794520102
39. Gheisari Y, Soleimani M, Azadmanesh K, Zeinali S. Multipotent mesenchymal stromal cells: optimization and comparison of five cationic polymer-based gene delivery methods. *Cytotherapy.* 2008;10(8):815–823. doi:10.1080/14653240802474307
40. Lu CH, Lin KJ, Chiu HY, et al. Improved chondrogenesis and engineered cartilage formation from TGF-beta3-expressing adipose-derived stem cells cultured in the rotating-shaft bioreactor. *Tissue Eng Part A.* 2012;18(19–20):2114–2124. doi:10.1089/ten.tea.2012.0010
41. Lo WH, Hwang SM, Chuang CK, Chen CY, Hu YC. Development of a hybrid baculoviral vector for sustained transgene expression. *Mol Ther.* 2009;17(4):658–666. doi:10.1038/mt.2009.13
42. He CX, Zhang TY, Miao PH, et al. TGF-beta1 gene-engineered mesenchymal stem cells induce rat cartilage regeneration using nonviral gene vector. *Biotechnol Appl Biochem.* 2012;59(3):163–169. doi:10.1002/bab.1001
43. Seo KW, Sohn SY, Bhang DH, Nam MJ, Lee HW, Youn HY. Therapeutic effects of hepatocyte growth factor-overexpressing human umbilical cord blood-derived mesenchymal stem cells on liver fibrosis in rats. *Cell Biol Int.* 2014;38(1):106–116. doi:10.1002/cbin.10186
44. Tan Z, Zhao Q, Gong P, et al. Research on promoting periodontal regeneration with human basic fibroblast growth factor-modified bone marrow mesenchymal stromal cell gene therapy. *Cytotherapy.* 2009;11(3):317–325. doi:10.1080/14653240902824757
45. Gandra N, Wang DD, Zhu Y, Mao C. Virus-mimetic cytoplasm-cleavable magnetic/silica nanoclusters for enhanced gene delivery to mesenchymal stem cells. *Angew Chem Int Ed Engl.* 2013;52(43):11278–11281. doi:10.1002/anie.201301113
46. Mun JY, Shin KK, Kwon O, Lim YT, Oh DB. Minicircle microporation-based non-viral gene delivery improved the targeting of mesenchymal stem cells to an injury site. *Biomaterials.* 2016;101:310–320. doi:10.1016/j.biomaterials.2016.05.057
47. Hubbs AF, Mercer RR, Benkovic SA, et al. Nanotoxicology - a pathologist's perspective. *Toxicol Pathol.* 2011;39(2):301–324. doi:10.1177/0192623310390705
48. Norppa H, Catalán J, Falck G, Hannukainen K, Siivola K, Savolainen K. Nano-specific genotoxic effects. *J Biomed Nanotechnol.* 2011;7(1):19. doi:10.1166/jbn.2011.1179
49. Fierro FA, Kalomoiris S, Sondergaard CS, Nolte JA. Effects on proliferation and differentiation of multipotent bone marrow stromal cells engineered to express growth factors for combined cell and gene therapy. *Stem Cells.* 2011;29(11):1727–1737. doi:10.1002/stem.720
50. Chen YA, Qian H, Zhu W, et al. Hepatocyte growth factor modification promotes the amelioration effects of human umbilical cord mesenchymal stem cells on rat acute kidney injury. *Stem Cells Dev.* 2011;20(1):103–113. doi:10.1089/scd.2009.0495
51. González MN, de Mello W, Butler-Brown GS, et al. HGF potentiates extracellular matrix-driven migration of human myoblasts: involvement of matrix metalloproteinases and MAPK/ERK pathway. *Skelet Muscle.* 2017;7(1):20. doi:10.1186/s13395-017-0138-6
52. Wang SX, Qin X, Sun DD, et al. Effects of hepatocyte growth factor overexpressed bone marrow-derived mesenchymal stem cells on prevention from left ventricular remodelling and functional improvement in infarcted rat hearts. *Cell Biochem Funct.* 2012;30(7):574–581. doi:10.1002/cbf.v30.7
53. Zhao MZ, Nonoguchi N, Ikeda N, et al. Novel therapeutic strategy for stroke in rats by bone marrow stromal cells and *ex vivo* HGF gene transfer from HSV-1 vector. *J Cereb Blood Flow Metab.* 2006;26(9):1176–1188. doi:10.1038/sj.cbfm.9600273

54. Moon HH, Joo MK, Mok H, et al. MSC-based VEGF gene therapy in rat myocardial infarction model using facial amphipathic bile acid-conjugated polyethyleneimine. *Biomaterials*. 2014;35(5):1744–1754. doi:10.1016/j.biomaterials.2013.11.019
55. Yanfu H, Ran T, Yanqing H, et al. Microencapsulated VEGF gene-modified umbilical cord mesenchymal stromal cells promote the vascularization of tissue-engineered dermis: an experimental study. *Cytotherapy*. 2014;16(2):160–169. doi:10.1016/j.jcyt.2013.10.014
56. Tee JK, Setyawati MI, Peng F, Leong DT, Ho HK. Angiopoietin-1 accelerates restoration of endothelial cell barrier integrity from nanoparticle-induced leakiness. *Nanotoxicology*. 2019;13(5):682–700. doi:10.1080/17435390.2019.1571646
57. Sun L, Cui M, Wang Z, et al. Mesenchymal stem cells modified with angiopoietin-1 improve remodeling in a rat model of acute myocardial infarction. *Biochem Biophys Res Commun*. 2007;357(3):779–784. doi:10.1016/j.bbrc.2007.04.010
58. Mei SH, McCarter SD, Deng Y, Parker CH, Liles WC, Stewart DJ. Prevention of LPS-induced acute lung injury in mice by mesenchymal stem cells overexpressing angiopoietin 1. *PLoS Med*. 2007;4(9):e269. doi:10.1371/journal.pmed.0040269
59. Xu J, Qu J, Cao L, et al. Mesenchymal stem cell-based angiopoietin-1 gene therapy for acute lung injury induced by lipopolysaccharide in mice. *J Pathol*. 2008;214(4):472–481. doi:10.1002/path.2302
60. Li Y, Zheng L, Xu X, et al. Mesenchymal stem cells modified with angiopoietin-1 gene promote wound healing. *Stem Cell Res Ther*. 2013;4(5):113. doi:10.1186/scrt324
61. Schabitz WR, Sommer C, Zoder W, Kiessling M, Schwaninger M, Schwab S. Intravenous brain-derived neurotrophic factor reduces infarct size and counterregulates Bax and Bcl-2 expression after temporary focal cerebral ischemia. *Stroke*. 2000;31(9):2212–2217. doi:10.1161/01.STR.31.9.2212
62. Wang Z, Yao W, Deng Q, Zhang X, Zhang J. Protective effects of BDNF overexpression bone marrow stromal cell transplantation in rat models of traumatic brain injury. *J Mol Neurosci*. 2013;49(2):409–416. doi:10.1007/s12031-012-9908-0
63. Kurozumi K, Nakamura K, Tamiya T, et al. BDNF gene-modified mesenchymal stem cells promote functional recovery and reduce infarct size in the rat middle cerebral artery occlusion model. *Mol Ther*. 2004;9(2):189–197.
64. Li WJ, Tuli R, Okafor C, et al. A three-dimensional nanofibrous scaffold for cartilage tissue engineering using human mesenchymal stem cells. *Biomaterials*. 2005;26(6):599–609.
65. Herrmann JL, Wang Y, Abarbanell AM, Weil BR, Tan J, Meldrum DR. Preconditioning mesenchymal stem cells with transforming growth factor- α improves mesenchymal stem cell-mediated cardioprotection. *Shock*. 2010;33(1):24–30.
66. Huang AH, Motlekar NA, Stein A, Diamond SL, Shore EM, Mauck RL. High-throughput screening for modulators of mesenchymal stem cell chondrogenesis. *Ann Biomed Eng*. 2008;36(11):1909–1921.
67. Sciolli MG, Cervelli V, Arcuri G, et al. High insulin-induced down-regulation of Erk-1/IGF-1R/FGFR-1 signaling is required for oxidative stress-mediated apoptosis of adipose-derived stem cells. *J Cell Physiol*. 2014;229(12):2077–2087.
68. Savi M, Bocchi L, Fiumana E, et al. Enhanced engraftment and repairing ability of human adipose-derived stem cells, conveyed by pharmacologically active microcarriers continuously releasing HGF and IGF-1, in healing myocardial infarction in rats. *J Biomed Mater Res A*. 2015;103(9):3012–3025.
69. Gomez-Mauricio G, Moscoso I, Martin-Cancho MF, et al. Combined administration of mesenchymal stem cells overexpressing IGF-1 and HGF enhances neovascularization but moderately improves cardiac regeneration in a porcine model. *Stem Cell Res Ther*. 2016;7(1):94.
70. Chen B, Li Q, Zhao B, Wang Y. Stem cell-derived extracellular vesicles as a novel potential therapeutic tool for tissue repair. *Stem Cells Transl Med*. 2017;6(9):1753–1758.
71. Choi JS, Yoon HI, Lee KS, et al. Exosomes from differentiating human skeletal muscle cells trigger myogenesis of stem cells and provide biochemical cues for skeletal muscle regeneration. *J Control Release*. 2016;222:107–115.
72. Tsuji K, Kitamura S, Wada J. Immunomodulatory and regenerative effects of mesenchymal stem cell-derived extracellular vesicles in renal diseases. *Int J Mol Sci*. 2020;21:3.
73. Tan CY, Lai RC, Wong W, Dan YY, Lim S-K, Ho HK. Mesenchymal stem cell-derived exosomes promote hepatic regeneration in drug-induced liver injury models. *Stem Cell Res Ther*. 2014;5(3):76.
74. Choi JS, Yoon HI, Lee KS, et al. Exosomes from differentiating human skeletal muscle cells trigger myogenesis of stem cells and provide biochemical cues for skeletal muscle regeneration. *J Control Release*. 2016;222:107–115.
75. Shafei AE, Ali MA, Ghanem HG, et al. Mesenchymal stem cells therapy: a promising cell based therapy for treatment of myocardial infarction. *J Gene Med*. 2017;19:12. doi:10.1002/jgm.2995
76. Ikeda N, Nonoguchi N, Zhao MZ, et al. Bone marrow stromal cells that enhanced fibroblast growth factor-2 secretion by herpes simplex virus vector improve neurological outcome after transient focal cerebral ischemia in rats. *Stroke*. 2005;36(12):2725–2730.
77. Pollock K, Dahlenburg H, Nelson H, et al. Human mesenchymal stem cells genetically engineered to overexpress brain-derived neurotrophic factor improve outcomes in Huntington's disease mouse models. *Mol Ther*. 2016;24(5):965–977.
78. Zhao GY, Cui L, Gao J, Dai RT, Zhang P. Study on the levels of DA and metabolite in striatum in rats with Parkinson's disease treated by BDNF gene modified bone mesenchymal stem cells. *Zhongguo Ying Yong Sheng Li Xue Za Zhi*. 2013;29(1):82–85.
79. Chen H, Xia R, Li Z, et al. Mesenchymal stem cells combined with hepatocyte growth factor therapy for attenuating ischaemic myocardial fibrosis: assessment using multimodal molecular imaging. *Sci Rep*. 2016;6:33700.
80. Gao F, He T, Wang H, et al. A promising strategy for the treatment of ischemic heart disease: mesenchymal stem cell-mediated vascular endothelial growth factor gene transfer in rats. *Can J Cardiol*. 2007;23(11):891–898.
81. Li Z, Mei J, Zhang B. Cell transplantation of 5-aza cytidine induced bone marrow stromal cells transfected by angiogenin gene ex vivo into infarcted myocardium, an experimental study. *Zhonghua Yi Xue Za Zhi*. 2002;82(19):1319–1323.
82. Fan L, Lin C, Zhuo S, et al. Transplantation with survivin-engineered mesenchymal stem cells results in better prognosis in a rat model of myocardial infarction. *Eur J Heart Fail*. 2009;11(11):1023–1030.
83. Wang H, Sun RT, Li Y, et al. HGF Gene Modification in mesenchymal stem cells reduces radiation-induced intestinal injury by modulating immunity. *PLoS One*. 2015;10(5):e0124420.
84. Hao L, Wang J, Zou Z, et al. Transplantation of BMSCs expressing hPDGF-A/hBD2 promotes wound healing in rats with combined radiation-wound injury. *Gene Ther*. 2009;16(1):34–42.
85. Xue J, Li X, Lu Y, et al. Gene-modified mesenchymal stem cells protect against radiation-induced lung injury. *Mol Ther*. 2013;21(2):456–465.
86. Huang JH, Surgery BDO, Hospital D, et al. Intravenous injection of nerve growth factor gene-modified bone marrow stromal stem cells on the apoptosis of the mouse hepatic cells induced by radiation. *J Clin Rehab Tissue Eng Res*. 2008;12(38):7503–7506.
87. Kumar S, Nagy TR, Ponnazhagan S. Therapeutic potential of genetically modified adult stem cells for osteopenia. *Gene Ther*. 2010;17(1):105–116.

88. Dulamea AO. The potential use of mesenchymal stem cells in stroke therapy—From bench to bedside. *J Neurol Sci.* **2015**;352(1–2):1–11.
89. Su YR, Wang J, Wu JJ, Chen Y, Jiang YP. Overexpression of lentivirus-mediated glial cell line-derived neurotrophic factor in bone marrow stromal cells and its neuroprotection for the PC12 cells damaged by lactacystin. *Neurosci Bull.* **2007**;23(2):67–74.
90. Lundberg J, Jussing E, Liu Z, et al. Safety of intra-arterial injection with tumor-activated T cells to the rabbit brain evaluated by MRI and SPECT/CT. *Cell Transplant.* **2017**;26(2):283–292.
91. Bhatia V, Gupta V, Khurana D, Sharma RR, Khandelwal N. Randomized assessment of the safety and efficacy of intra-arterial infusion of autologous stem cells in subacute ischemic stroke. *AJNR.* **2018**;39(5):899–904.
92. Feng CJ, Peng CK, Lin CH, Tsai CH, Huang PH, Ma H. Intra-arterial injection of human adipose-derived stem cells improves viability of the random component of axial skin flaps in nude mice. *J Plast Reconstr Aesthet Surg.* **2020**;73(3):598–607.
93. Di Spigna G, Iannone M, Ladogana P, et al. Human cardiac multipotent adult stem cells in 3D matrix: new approach of tissue engineering in cardiac regeneration post-infarction. *J Biol Regul Homeost Agents.* **2017**;31(4):911–921.
94. Tang YL, Tang Y, Zhang YC, Qian K, Shen L, Phillips MI. Improved graft mesenchymal stem cell survival in ischemic heart with a hypoxia-regulated heme oxygenase-1 vector. *J Am Coll Cardiol.* **2005**;46(7):1339–1350.
95. Liu N, Zhang Y, Fan L, et al. Effects of transplantation with bone marrow-derived mesenchymal stem cells modified by Survivin on experimental stroke in rats. *J Transl Med.* **2011**;9:105.
96. Li W, Ma N, Ong LL, et al. Bcl-2 engineered MSCs inhibited apoptosis and improved heart function. *Stem Cells.* **2007**;25(8):2118–2127.
97. Mangi AA, Noiseux N, Kong DL, et al. Mesenchymal stem cells modified with Akt prevent remodeling and restore performance of infarcted hearts. *Nat Med.* **2003**;9(9):1195–1201.
98. Gnecci M, He H, Noiseux N, et al. Evidence supporting paracrine hypothesis for Akt-modified mesenchymal stem cell-mediated cardiac protection and functional improvement. *FASEB J.* **2006**;20(6):661–669.
99. Gallo S, Sala V, Gatti S, Crepaldi T. Cellular and molecular mechanisms of HGF/Met in the cardiovascular system. *Clin Sci (Lond).* **2015**;129(12):1173–1193.
100. Forte G, Minieri M, Cossa P, et al. Hepatocyte growth factor effects on mesenchymal stem cells: proliferation, migration, and differentiation. *Stem Cells.* **2006**;24(1):23–33. doi:10.1634/stemcells.2004-0176
101. Lu F, Zhao X, Wu J, et al. MSCs transfected with hepatocyte growth factor or vascular endothelial growth factor improve cardiac function in the infarcted porcine heart by increasing angiogenesis and reducing fibrosis. *Int J Cardiol.* **2013**;167(6):2524–2532. doi:10.1016/j.ijcard.2012.06.052
102. Gomez-Mauricio G, Moscoso I, Martin-Cancho MF, et al. Combined administration of mesenchymal stem cells overexpressing IGF-1 and HGF enhances neovascularization but moderately improves cardiac regeneration in a porcine model. *Stem Cell Res Ther.* **2016**;7(1):94. doi:10.1186/s13287-016-0350-z
103. Yang ZJ, Ma DC, Wang W, et al. Experimental study of bone marrow-derived mesenchymal stem cells combined with hepatocyte growth factor transplantation via noninfarct-related artery in acute myocardial infarction. *Gene Ther.* **2006**;13(22):1564–1568. doi:10.1038/sj.gt.3302820
104. Zhang J, Wang LL, Du W, et al. Hepatocyte growth factor modification enhances the anti-arrhythmic properties of human bone marrow-derived mesenchymal stem cells. *PLoS One.* **2014**;9(10):e111246. doi:10.1371/journal.pone.0111246
105. Laddha AP, Kulkarni YA. VEGF and FGF-2: promising targets for the treatment of respiratory disorders. *Respir Med.* **2019**;156:33–46. doi:10.1016/j.rmed.2019.08.003
106. Giri TK, Alexander A, Agrawal M, Saraf S, Saraf S. Ajazuddin. Current status of stem cell therapies in tissue repair and regeneration. *Curr Stem Cell Res Ther.* **2019**;14(2):117–126. doi:10.2174/1574888X13666180502103831
107. Kong F, Shi X, Xiao F, et al. Transplantation of HGF modified dental pulp stem cells prevents bone loss in the early phase of ovariectomy-induced osteoporosis. *Hum Gene Ther.* **2018**;29(2):271–282. doi:10.1089/hum.2017.091
108. Zhang J, Chen J. Bone tissue regeneration - application of mesenchymal stem cells and cellular and molecular mechanisms. *Curr Stem Cell Res Ther.* **2017**;12(5):357–364. doi:10.2174/1574888X11666160921121555
109. Pacelli S, Basu S, Whitlow J, et al. Strategies to develop endogenous stem cell-recruiting bioactive materials for tissue repair and regeneration. *Adv Drug Deliv Rev.* **2017**;120:50–70. doi:10.1016/j.addr.2017.07.011
110. Brunger JM, Huynh NP, Guenther CM, et al. Scaffold-mediated lentiviral transduction for functional tissue engineering of cartilage. *Proc Natl Acad Sci U S A.* **2014**;111(9):E798–E806. doi:10.1073/pnas.1321744111
111. Wang T, Liao T, Wang H, Deng W, Yu D. Transplantation of bone marrow stromal cells overexpressing human vascular endothelial growth factor 165 enhances tissue repair in a rat model of radiation-induced injury. *Chin Med J (Engl).* **2014**;127(6):1093–1099.
112. Hu S, Chen Y, Li L, et al. Effects of adenovirus-mediated delivery of the human hepatocyte growth factor gene in experimental radiation-induced heart disease. *Int J Radiat Oncol Biol Phys.* **2009**;75(5):1537–1544. doi:10.1016/j.ijrobp.2009.07.1697
113. Li Q, Sun H, Xiao F, et al. Protection against radiation-induced hematopoietic damage in bone marrow by hepatocyte growth factor gene transfer. *Int J Radiat Biol.* **2014**;90(1):36–44. doi:10.3109/09553002.2014.847294
114. Zhang J, Zhou S, Zhou Y, et al. Hepatocyte growth factor gene-modified adipose-derived mesenchymal stem cells ameliorate radiation induced liver damage in a rat model. *PLoS One.* **2014**;9(12):e114670. doi:10.1371/journal.pone.0114670
115. Chen S, Chen X, Wu X, et al. Hepatocyte growth factor-modified mesenchymal stem cells improve ischemia/reperfusion-induced acute lung injury in rats. *Gene Ther.* **2017**;24(1):3–11. doi:10.1038/gt.2016.64
116. Shao Y, Shen J, Zhou F, He D. Mesenchymal stem cells overexpressing Ang1 attenuates phosgene-induced acute lung injury in rats. *Inhal Toxicol.* **2018**;30(7–8):313–320. doi:10.1080/08958378.2018.1521483
117. Sage EK, Thakrar RM, Janes SM. Genetically modified mesenchymal stromal cells in cancer therapy. *Cytotherapy.* **2016**;18(11):1435–1445. doi:10.1016/j.jcyt.2016.09.003
118. Weiss DJ, Casaburi R, Flannery R, LeRoux-Williams M, Tashkin DP. A placebo-controlled, randomized trial of mesenchymal stem cells in COPD. *Chest.* **2013**;143(6):1590–1598. doi:10.1378/chest.12-2094
119. Wilson JG, Liu KD, Zhuo H, et al. Mesenchymal stem (stromal) cells for treatment of ARDS: a phase 1 clinical trial. *Lancet Respir Med.* **2015**;3(1):24–32. doi:10.1016/S2213-2600(14)70291-7
120. Ripa RS, Wang Y, Jørgensen E, Johnsen HE, Hesse B, Kastrup J. Intramyocardial injection of vascular endothelial growth factor-A165 plasmid followed by granulocyte-colony stimulating factor to induce angiogenesis in patients with severe chronic ischaemic heart disease. *European Heart Journal.* **2006**;27(15):1785–1792.
121. Wheelock V, Tempkin T, Duffy A, et al. PRE-CELL: clinical and novel biomarker measures of disease progression in a lead-in-observational study for a planned phase 1 trial of genetically modified mesenchymal stem cells over-expressing BDNF in patients with Huntington's disease (S25.004). *Neurology.* **2016**;86(16 Supplement):S25.004.

122. Deng P, Torrest A, Pollock K, et al. Clinical trial perspective for adult and juvenile Huntington's disease using genetically-engineered mesenchymal stem cells. *Neural Regen Res*. 2016;11(05):702–705. doi:10.4103/1673-5374.182682
123. Daley GQ, Hyun I, Apperley JF, et al. Setting global standards for stem cell research and clinical translation: the 2016 ISSCR guidelines. *Stem Cell Reports*. 2016;6(6):787–797. doi:10.1016/j.stemcr.2016.05.001
124. Munoz Ruiz M, Regueiro JR. New tools in regenerative medicine: gene therapy. *Adv Exp Med Biol*. 2012;741:254–275.
125. Poulos J. The limited application of stem cells in medicine: a review. *Stem Cell Res Ther*. 2018;9(1):1. doi:10.1186/s13287-017-0735-7
126. Imamura A, Kajiya H, Fujisaki S, et al. Three-dimensional spheroids of mesenchymal stem/stromal cells promote osteogenesis by activating stemness and Wnt/beta-catenin. *Biochem Biophys Res Commun*. 2020;523(2):458–464. doi:10.1016/j.bbrc.2019.12.066
127. King NM, Perrin J. Ethical issues in stem cell research and therapy. *Stem Cell Res Ther*. 2014;5(4):85. doi:10.1186/sct474

Drug Design, Development and Therapy

Dovepress

Publish your work in this journal

Drug Design, Development and Therapy is an international, peer-reviewed open-access journal that spans the spectrum of drug design and development through to clinical applications. Clinical outcomes, patient safety, and programs for the development and effective, safe, and sustained use of medicines are a feature of the journal, which has also

been accepted for indexing on PubMed Central. The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <https://www.dovepress.com/drug-design-development-and-therapy-journal>