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REVIEW

Cellular mechanisms of emerging applications of mesenchymal stem cells

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Abstract

Mesenchymal stem cells (MSC) are multipotent, self-renewing cells that can be found mainly in the bone marrow, and other post-natal organs and tissues. The ease of isolation and expansion, together with the immunomodulatory properties and their capability to migrate to sites of inflammation and tumours make them a suitable candidate for therapeutic use in the clinical settings. We review here the cellular mechanisms underlying the emerging applications of MSC in various fields.

Keywords: mesenchymal stem cells, cancer, nanoparticle, regenerative medicine, cell-based therapy

INTRODUCTION

Mesenchymal stem cells (MSC) are multipotent, self-renewing cells that reside mainly in the bone marrow. It is believed that MSC are remnants of embryonic stem cells (ESC) that persist in adult life as they exhibit ESC genetic markers of Oct-4, Rex-1, Nanog, GATA-4 and SSEA-1.[1,2] MSC were hypothesized to be re-distributed via the blood stream to other organs or tissues to maintain stem cells homeostasis in a microenvironment or mobilized upon receiving chemokine signals released by damaged organs and tissues for regenerative purposes.[3]

During tissue injury, released cytokines/growth factors or reactive oxygen species (ROS) will activate the expression of endothelial receptors such as P-selectin or intercellular adhesion molecule 1 (ICAM-1) in the blood vessels. Since MSC express various cytokine and growth factor receptors on their membrane surfaces, they are likely to migrate from their site of residence towards cytokine / growth factor production sites by sensing these cytokine gradients. The free travelling MSC in the blood stream can bind or adhere to the receptors on the activated endothelial cells. The tethering and rolling will decelerate the cells from the blood stream and allow trans-endothelial migration into the tissue in a similar manner to leukopedesis.[4,5] Cells which fail to transmigrate are usually embedded in the layers of endothelium in the blood vessel (Figure 1).

The function of MSC as perivascular cells, most commonly referred as pericytes,[6] in settings of focal injury has been outlined by Caplan.[7] It is postulated that MSC could secrete large amounts of bioactive molecules that contribute to immunomodulatory functions and, re-structuring of a suitable microenvironment for preparation of new tissue regeneration or tissue repair. The immunomodulatory activity can protect the injury site from immune surveillance by suppressing T-cells activation and proliferation. Also, by dampening chronic inflammatory activity, MSC can reduce further apoptosis due to ischaemia, inhibit the recruitment of fibroblasts, and therefore result in less scar formation. The bioactive molecules also stimulate the mitosis and differentiation of local progenitor stem cells to reform the damaged tissue. By residing in the vascular wall, MSC may also stimulate and stabilize angiogenesis and vessel reformation. MSC may also regenerate new tissue by cell differentiation or fusion.

This ability to migrate to sites of tissue injury, for example, bone fracture, cerebral ischaemia and infarcted heart,[8,9] in addition to the ease of isolation and expansion, and amenability to genetic engineering have made MSC a suitable candidate for cellular therapy.[10] The International

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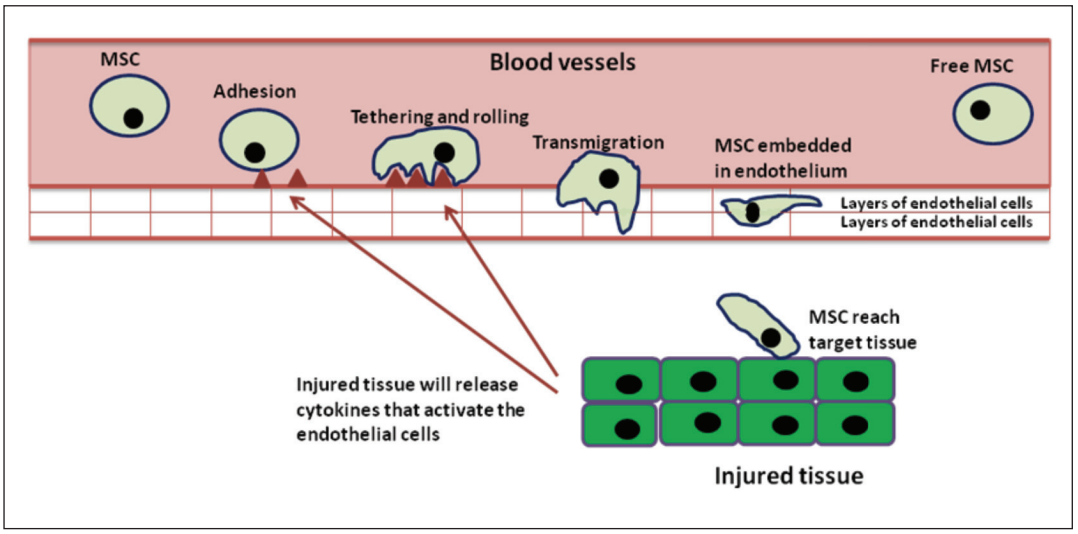

FIG. 1: MSC migration to the site of tissue injury. Damaged tissues release chemoattractants or reactive oxygen species which can activate the expression of receptors, such as P-selectin or ICAM-1 on the endothelial cells in the blood vessel. As MSC travel in the blood stream, MSC could recognize and adhere onto the endothelial receptors. Following adherence, MSC would transmigrate across the endothelium to reach the damaged tissues. Cells which fail to transmigrate would be embedded in the endothelial layers.

Society for Cellular Therapy has defined MSC as capable of adhering to plastic flask when maintained in standard culture conditions. Second, 95% of the MSC population must express CD105, CD73 and CD90, as measured by flow cytometry. Additionally, these cells must lack expression (≤ 2% positive) of CD45, CD34, CD14 or CD11b, CD79a or CD19 and HLA class II. Third, the cells must be able to differentiate to osteoblasts, adipocytes and chondroblasts.[11] Other than the bone marrow, MSC could also be found in umbilical cord,[12] amniotic fluid,[13] peripheral blood,[14] fallopian tube,[15] cornea,[16] adipose,[17] synovial tissue,[18] dental pulp,[19] placenta,[20] bone,[21] liver[22] and lung.[23] Other sources of human MSC include the intestinal, limbal, knee-joint and prostate stromas, trachea and nasal mucosae.[24]

The immunomodulatory properties of MSC

Cell-based therapy using MSC are more advantageous over other cell types as they are considered 'immunoprivileged'. This 'veto' function can be ascribed to the lack of major histocompatibility class II (MHCII), and CD80 (B7-1), CD86 (B7-2) and CD40 co-stimulatory antigen expression on MSC. Despite pre-incubation with interferon-γ (IFN-γ) for full human leucocyte antigen (HLA) class II expression, MSC could still escape recognition by alloreactive T-cells. The engagement of CD28 expressed on T-cells with B7-1 and / or B7-2 expressed on a variety of cell types assist in co-stimulating T-cells activation via T-cell receptor (TCR).[25] However, provision of CD28-mediated co-stimulation[26] or transduction of B7-1 or B7-2 genes into MSC,[27] was still unsuccessful to reverse the failure for T-cells activation.[2]

There are increasing evidences showing that MSC promote the conversion from a Th1 immune to a Th2 immune response as a net effect of complex regulation of co-stimulatory molecules and cytokines expressed by both MSC and immune cells (Figure 2). In *in vitro* studies, T-cells could be stimulated when incubated with mitogens or allogeneic T-cells in mixed lymphocyte reactions (MLRs) or by engagement of TCR and its co-stimulatory receptors. However, in the presence of MSC, the proliferation of CD3+/CD4+ cells and secretion of Th1 lymphokines such interleukin-2 (IL-2) and interferon-γ (IFN-γ) were suppresed.[28] The suppression of both lymphokines could inhibit the differentiation of naïve CD8+ cells into cytotoxic effectors.[29] Also, Sato and his colleagues have reported that incubation with T-cells could trigger the production and release of nitric oxide from MSC, which will in turn, suppress the proliferating T-cells.[30] Unlike *in vivo* use of MSC for therapeutic indications, these observations require priming of MSC to IFN-γ.

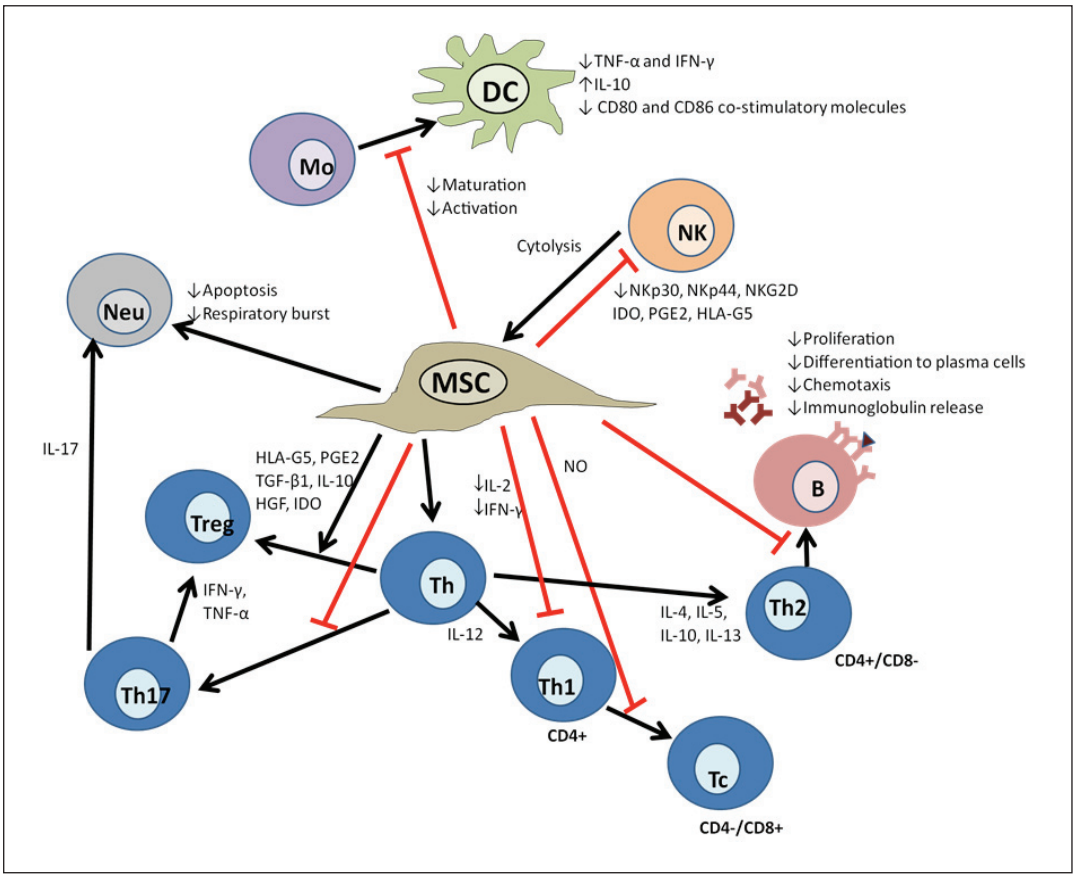

FIG. 2: Immunomodulation of MSC. A summary of the range of soluble and cell surface proteins that may mediate the effects of human MSC on immune responses. Abbreviations: MSC, mesenchymal stem cell; Th, helper T-cell; Tc, cytotoxic T-cell; B, B-cell; Treg, regulatory T-cell; Neu, neutrophil; Mo, monocyte; DC, dendritic cell; NK, natural killer cell; NO, nitric oxide; IFN-γ; interferon γ; TNF-α, tumour necrosis factor-α; PGE2, prostaglandin E2; TGF-β1, transforming growth factor-β1; IL, interleukin; HGF, hepatocyte growth factor; IDO, indoleamine 2,3-dioxygenase; HLA-G5, human leukocyte antigen-G5; NKp30, natural killer cell p-30 related protein; NKp44, natural killer p-44 related protein; NKG2D, natural killer group 2, member D.

Upon intercellular contact with activated CD4+ cells, MSC can up-regulate the expression of HLA-G5 molecule which will then trigger the activated CD4+ cells to differentiate into Treg cells (CD4+/CD25+/Foxp3+). Other bioactive molecules secreted by MSC such as prostaglandin E2 (PGE2), transforming growth factor-β1 (TGF-β1), interleukin-10 (IL-10), hepatocyte growth factor (HGF) and indoleamine 2,3-dioxygenase (IDO) could also induce the differentiation of Treg cells.[31] Treg cells mediate immunological tolerance and therefore might be manipulated for treating inflammation and reducing risk of rejection in allogeneic cell, tissue or organ transplantation. Another mechanism which MSC can help to harness immunological tolerance is by directly inhibiting *in vitro* differentiation of naïve CD4+ into Th17 cells. This subset of cells produce high levels of IL-17 and are an important effector cells in host defence against certain pathogens such as *Candida albicans* and specific extracellular bacteria. The receptors for IL-17 are widely present in many tissues and might be responsible for tissue inflammation and autoimmunity upon Th17 cells differentiation.[32] Ghannam *et al*[33] has also shown the capability of MSC to induce Treg cell function in the Th17 cells in the presence of IFN-γ and TNF-α. When MSC were co-cultured with Th17 cells, the production of PGE2, which was already constitutively expressed in MSC, was further enhanced and thus added to the suppressive effect of MSC. In contrary, Guo *et al* [34] found that foetal bone marrow MSC could promote the

expansion of Th17 cells instead. There was also a report demonstrating that MSC could induce higher secretion of IL-17 following contact with activated T-cells *in vitro*.[35]

MSC may also interact and regulate the immune response of B lymphocytes. It is reported that in a co-culture of bone marrow MSC and stimulated B-cells from peripheral blood of healthy donors, the proliferation of B-cells and immunoglobulin production (IgG, IgA and IgM) were inhibited. The chemotactic property of B-cells was also believed to be affected since chemokine (C-X-C motif) receptors (CXCR4 and CXCR5), and the ligands of CXCR4 and CXC12 were significantly down-regulated by MSC.[36] In contrast, other reports have shown that MSC are capable of inducing immature transitional and naïve B-cells to undergo proliferation and differentiation into antibody-secreting cells in response to polyclonal stimuli even in the absence of antigen,[37] and immunoglobulin G production from B-cells in a mononuclear cell population.[38] These mechanisms may be attributed to the progression of B-cell-mediated disease such as multiple myeloma, rheumatoid arthritis and systemic lupus erythematosus.[39,40]

MSC have also been suggested to play an important role in bridging adaptive and innate immune responses. MSC can dampen respiratory burst in activated neutrophils and protect neutrophils from apoptosis without impairing their phagocytic and chemotactic activity.[41] However, according to Hsu *et al,*[35] following contact between MSC and CD4+/CD45+/RO+ T-cells, the release of IL-17 could increase the phagocytic activity of neutrophils *in vitro*. It was also reported that MSC inhibited NK cell-mediated cytolysis and IFN-γ secretion by producing soluble mediators such as indoleamine 2,3-dioxygenase (IDO), PGE2 and HLA-G.[42,43] At low ratios of natural killer (NK) cell to MSC, MSC could down-regulate the expression of NKp30, NKp44 and NKG2D, surface receptors involved in NK cell activation. Since MSC express MHCI, MSC were also susceptible to lysis by activated NK cells.[44] Meanwhile, when MSC were cultured with monocytes, monocytes failed to differentiate into dendritic cells (DC) by inhibiting its maturation and function to present antigen to T-cells via CD80, CD86 and IL-12. The inhibitory effect of MSC on DC differentiation might be mediated through activation of the Notch signalling pathway. DC were ineffective in their ability to activate lymphocytes by suppressing TNF-α and IFN-γ and upregulating IL-10 expression in DC-CD4+ MLRs.[28,45,46]

The reparative mechanisms

Early use of MSC for treatment has exploited on the differentiation properties of MSC to specific cells to replace damaged cells as evidenced by both *in vitro* and *in vivo* studies. The differentiated cells derived from MSC, such as osteogenic cells were used for osteogenic imperfecta patients,[47,48] chondrogenic cells for knee cartilage repair,[49] mesangial cells for repair of post-glomerular injury[50] and myocardiocytes for heart regeneration.[51] MSC also exhibit trans-differentiation potential toward endodermal hepatic[52] and neuronal lineage cells.[53] The stem cell division and differentiation into lineage specific cells were processes controlled by a combination of the intrinsic fluctuations of protein concentrations and gene state fluctuations through promoter binding.[54] These conditions can be influenced by the environment, including growth factors or chemical agents,[55] mechanical or physical stimulation,[56,57] cell-cell attachment or interactions, cell density[58,59] or direct introduction of regulatory genes into the cells.[60]

MSC have also been found to exert reparative effect in ischaemically damaged cardiomyoblast through cell fusion. Bone marrow-derived stem cell fusion has earlier been implicated in the rescue of skeletal muscle and hepatic cells too.[61] During cell fusion, the cells connect and exchange vital cell components via nanotubes.[62,63] Within the central nervous system (CNS), fusion of MSC and Purkinje cells has been observed and rarely occur under normal biological conditions. In a damage to CNS, inflammation could promote migration and infiltration of MSC to the site of injury and thereby, increase the frequency of stem cell fusion as a means of neuroprotection or rescue of highly differentiated cell types which are not replaceable in adults, and to limit the loss of structural neurons such as Purkinje cells.[64] In a recent finding, MSC was found to have acquired epithelial characteristics through fusion with gastrointestinal epithelial cells and might play an important role to quickly replace the shed epithelial cells from mucosal lining.[65]

However, recent clinical trials have focused on the ability of MSC to exert their biological function through the paracrine and endocrine actions of the secreted cytokines. This shift of idea was based on the observations that MSC therapy resulted in reduction of inflammation, apoptosis, fibrosis and enhanced angiogenesis

despite a lack of MSC engraftment and differentiation in numerous disease models. It is also possible that the trophic factors stimulate the local progenitor stem cells to divide and differentiate into functional cells.[66-70] It is shown that treatment with MSC-conditioned medium alone has resulted in reduced size of infarct and preserved diastolic and systolic function of the heart in a porcine model of myocardial infarction,[71] and enhanced angiogenesis and fracture healing in a diabetic rat model.[72] Conditioned medium from MSC culture could also inhibit corneal fibroblast proliferation and migration during corneal wound healing, and improve corneal clarity.[73,74]

It is important to note that MSC may exert its reparative property using more that one of the suggested pathways (Figure 3). In wound healing, for example, there are evidences that MSC differentiate into epidermal keratinocytes, endothelial cells, pericytes and sebocytes in the sebaceous glands in skin adjacent to the wound *in vivo*. Meanwhile, MSC paracrine signalling enhances the migration, recruitment and proliferation of epidermal keratinocytes and dermal fibroblasts to accelerate wound closure. Co-culture of MSC or MSC-conditioned medium have both up-regulated the secretion of collagen type I from dermal fibroblasts and therefore, suggested a possible role of MSC to indirectly remodel the extracellular matrix at the site of wound.[75]

Current state of clinical trials and future trend in MSC application in medicine

Clinical trials using MSC have targeted on a broad spectrum of diseases involving the bone, cartilage, heart, lung, liver, kidney, pancreas, skin, gastrointestinal tract, islet cells, nerve cells, connective tissues and graft rejection.[76-79] The state of clinical trials has been extensively reviewed by Ankrum and Karp,[80] and the results have both been very promising and discouraging. For example, while early clinical trials established a good record of safety for direct MSC injection, MSC therapy has failed to achieve primary endpoints as there was no significant improvement in patients compared with control groups in both phase III graft versus host disease (GvHD) and phase II chronic obstructive pulmonary disease (COPD) trials. The phase III trial study on the efficiency of MSC to treat Crohn's disease has been ceased in 2009 as there was a greater-than-expected placebo response.[28] These results may indicate an incomplete understanding of the mechanisms of action of MSC, and thus preparation and administration of cells were not able to be employed efficiently in order to deliver therapeutic benefits. It is noteworthy that the culture environment which MSC are subjected to (e.g. hypoxic *vs.* normoxic and adherence *vs.* non-adherence), the timing and tissue site specificity of MSC delivery will all affect treatment efficacy.[81,82]

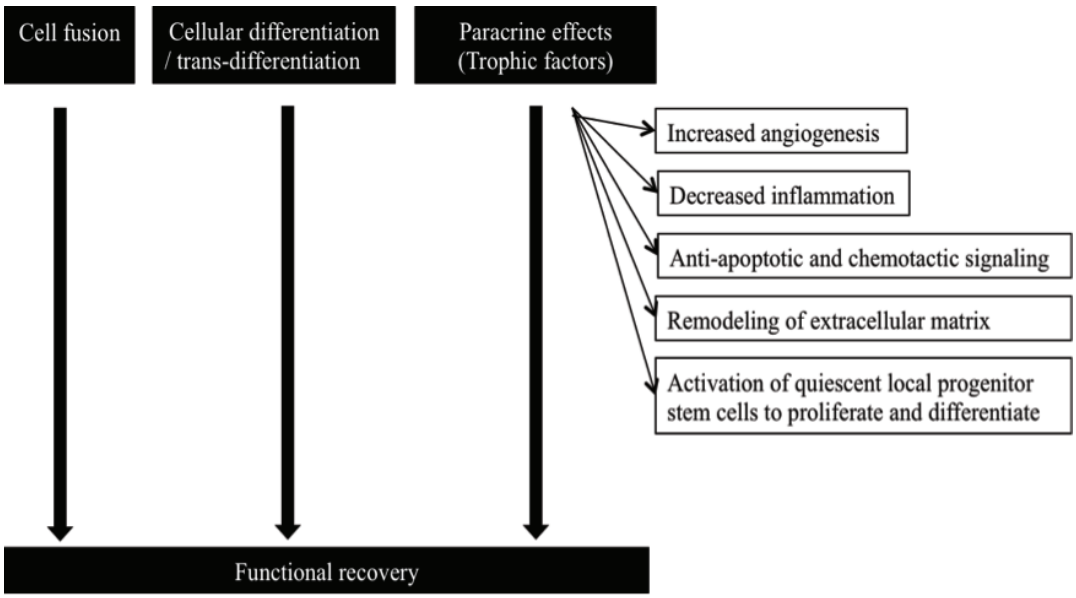

FIG. 3: The reparative mechanisms of MSC to improve tissue functions.

Emerging application of mesenchymal stem cells in gene therapy

Despite the lack of understanding and mixed outcomes, researches on MSC continue to flourish. One of the potential areas is to manipulate MSC to deliver therapeutic gene, for example erythropoietin (EPO) gene, to correct anaemia in chronic kidney failure (CKF) patients. The use of stem cells in gene delivery is ideal because transfected gene could result in long-term EPO protein expression. Treatment with genetically-engineered MSC could reduce the risk of infection, development of pure red-cell aplasia and cost associated with long-term treatment regiment involving repetitive recombinant EPO (rhuEPO) injections.[83]

The abundant presence of EPO receptor found in extra-hematopoietic tissues has led to a potentially wider application of EPO gene-transfected MSC.[84,85] In animal studies, rhuEPO promotes functional recovery and enhances nerve regeneration after transection of sciatic nerve,[86] and stroke in neonates.[87] The neuroprotective properties of rhuEPO against injury caused by ischaemia, trauma, chemical toxicity, diabetes, and hypothermia in animal models and its underlying mechanisms of action have been reviewed by Ghezzi and Brines (2004).[88] However, it is important to note that owing to challenges to cross the blood-brain barrier in these experimental models, including a phase II clinical trial of human stroke, the systemic administration of rhuEPO generally require a higher dosage compared to the doses currently employed for treating anaemia.[89] Although acute systemic administration of rhuEPO for treatment of tissue injury is less likely to be harmful, long term use of rhuEPO for chronic diseases associated with neuronal apoptosis or neuroinflammation, such as Parkinson's disease, Alzheimer disease or multiple sclerosis, might lead to hypertension and thrombosis.[90-92]

Thus, the use of MSC as a vehicle to deliver EPO protein locally might serve as an ideal treatment for targeted tissue repair. There are evidences that the co-treatment of rhuEPO and MSC were more potent in treating cerebral ischaemia, as demonstrated by reduced lesion volume, increased cellular proliferation and neurogenesis, compared with single treatment with rhuEPO or MSC alone in Sprague-Dawley rats. These changes were also accompanied by improved mnesic performances.[93] EPO protein may act to trigger phosphorylation of janus associated kinase-2 (JAK2) and extracellular signal-regulated kinase 1/2 (ERK1/2) pathways in MSC and therefore, enhance the survivability of transplanted MSC in a hypoxic tissue.[94] Avoidance of invasive surgery involving the brain seems possible as there was a recent publication showing successful engraftment and survivability of MSC in the brain delivered via intranasal route.[95]

Stem cell-based gene therapies also hold promise for treating a disease *in utero* caused by a genetic defect. By providing *in utero* therapy, a disease can be successfully treated before debilitating manifestations appear, and there is less risk of foreign cells rejection since the immune system of the foetus is not fully developed. There is also less scarring as a result of *in utero* correction of structural congenital abnormalities such as spina bifida. Moreover, owing to the small size of a foetus, a relatively high dosage of cells can be delivered, making the therapy more likely to succeed.[96,97]

There are a few proof-of-concept experiments involving use of MSC for foetal gene therapy which showed that intra-uterine stem cell transplantation (IUSCT) worked.[98-100] In human, Le Blanc had injected allogeneic foetal liver-derived MSC (fMSC) in the foetus with a severe non-lethal osteogenesis imperfect (OI) phenotype at 32 weeks of gestation. The patient had a surprisingly benign course and a minute number of osteoblasts engraftment was seen following the treatment.[101] Westgren has also reported a similar outcome in one of his unpublished data.[99] Despite the low frequency of donor osteoblast engraftment, the benefit of foetal liver-derived MSC for OI is encouraging.

The finding of stem cells in the amniotic fluid (AFSC) exhibiting characteristic properties similar to MSC is appealing as AFSC are an important source of autologous cells, and could offer another alternative to the use of fMSC for pre-natal and post-natal transplantation.[102] Autologous transplantation with AFSC can avoid the difficult process of finding a MHC-matched donor for treatment. The success rate of AFSC isolation was high and a number of $180x10^6$ cells could be easily expanded within 4 weeks (three passages) from a single 2 ml amniotic fluid sample. In comparison to that of bone marrow-derived MSC, AFSC showed greater proliferative and clonogenic potential (doubling time 25-38 h and 86±4.3 colonies *vs.* doubling time 30-90 h and 70±5.1 colonies). The AFSC retained a normal karyotype and displayed

no sign of tumourigenic potential even after extensive expansion in the laboratory .[97]

Emerging application using nanotechnology

The convergence of nanotechnology with stem cells has paved another new landscape in therapeutic medicine, specifically in the targeting and killing of cancers. The use of nanoparticles-labelled MSC for cancer therapy is advantageous as MSC could home to distant metastases[103-106] and allow simultaneous cancer detection using non-invasive imaging techniques e.g. magnetic resonance imaging, magnetic resonance spectroscopy, optical bioluminescence, optical fluorescence, targeted ultrasound, single photon emission computed tomography and positron emission tomography.[107-112] The use of magnetic resonance imaging to track iron-labelled MSC to lung metastases in animal model was first demonstrated by Loebinger and colleagues in 2009.[113] Therefore, anti-cancer drugs can be tethered to the nanoparticles[114] and delivered by MSC specifically to tumour sites, and this will overcome current limitations with free therapeutic nanoparticles including lack of water solubility and nonspecific bio-distribution due to leakage through blood vessel wall.[115] The uptake of nanoparticles such as gold,[116] polylactic acid,[117] superparamagnetic iron oxide and mesoporous silica[109] was found not to impair the viability, proliferation and differentiation functions of MSC. The release of anti-cancer drugs from nanomaterial can be controlled by an externally applied magnetic field at targeted tumour site.[118] These types of nanomaterial can also convert absorbed photons (Near Infrared {NIR} light) into high thermal energy and effectively kill tumour cells.[119-122] A novel work by Hong *et al*[123] has clearly shown the feasibility of photothermotherapy based on porous silicon in combination with NIR laser irradiation in selectively destroying tumour cells without damaging surrounding healthy cells.

MSC could home and engraft on atherosclerosis plaque.[124,125] The detonation of nanoshells by NIR irradiation could therefore burn away the arterial plaque and lysis of MSC can release cytokines or growth factors beneficial for preventing inflammation and help restoring the damaged artery.[126] In one study, silica-gold nanoshells were infused into the heart of transgenic swines along with MSC. The researchers found that the plaque volume shrunk in the test group immediately after the procedure and six months later, observed sign of neovascularization and restoration of artery function. On the other hand, nanoshells were less effective at eliminating plaque if not combined with MSC. In the control group that received only saline, the plaque volume increased an average of 4.3% instead.[127] This novel method seemed to really demolish the plaque and these promising results has led the researchers to submit for a phase I clinical trial (ClinicalTrials.gov Identifier NCT01270139) in September 2011. The final data collection for the primary outcome measure has been completed recently in patients with coronary artery disease (CAD) and SYNTAX (Synergy between PCI with Taxus and Cardiac Surgery) score that equals or less than 22, in June 2012. The results showed a significant regression of coronary atherosclerosis in patients receiving implanted bioengineered on-artery patch which was grown with allogeneic stem cells pre-cultivated with silica-gold NP in medium (n=60) versus stent implantation (n=60) at twelve months. The analysis of the event free survival of the ongoing clinical follow-up showed a significantly lower risk of cardiovascular death in this group compared with the control group (91.7% *vs*. 80%; $p<0.05$).[128]

Emerging application of mesenchymal stem cells in genitourinary and sexual medicine

Emerging application of MSC in the field of genitourinary and sexual medicine has also been observed lately. Worldwide, millions of women suffer from pelvic floor disorders including stress urinary incontinence (SUI), pelvic organ prolapse and bowel dysfunction. In a study carried out in the Cleveland Clinic, intravenously injected MSC was found to migrate to pelvic organs after simulated childbirth injury in female rats. This result suggests early intervention by MSC infusion during child delivery might be useful to repair possible injuries to the levator ani muscles and both urethral and ani sphincters for prevention of future pelvic floor disorders.[129,130] Meanwhile, Lin and his team have induced SUI in a rat model and evaluated the capability of MSC derived from fat pads of rat ovaries to mitigate voiding dysfunction. The efficiency of MSC delivered by intraurethral injection to mitigate voiding function was almost comparable to intravenous injection. As only a small fraction of MSC differentiated into smooth muscle cells, they suggested that the therapeutic effects of MSC were probably mediated by trophic factors that promote host tissue regeneration.[131]

Erectile dysfunction (ED) is used to describe a condition in men of their inability to achieve and

/ or maintain penile erection sufficient to allow satisfactory sexual intercourse, and associated with hypertension, diabetes, atherosclerosis, alcoholism, smoking, and pelvic surgery. Penile erection is achieved when the corporal smooth muscle is relaxed and blood accumulates in the penile tissue as a result of increase in arterial inflow and restriction of the venous outflow.[132,133] In 2007, Bivalacqua and his colleagues[134] injected endothelial nitric oxide synthase (eNOS)-transduced rat marrow MSC into the aged rat penile tissue and observed an improvement in the erectile response to cavernous nerve stimulation after one week. The eNOS protein is involved in the synthesis of NO, an important mediator of relaxation of corporal smooth muscle. Both genetically- and non-modified control MSC were found to have engrafted and differentiated into endothelial and smooth muscle in the corpora cavernosa. Interestingly, they found that the erectile response was also improved in the control group later on the third week and suggested that the MSC could have secreted certain trophic factors to influence erectile mechanism. In a similar study,[135] MSC transduced to secrete vascular endothelial growth factor (VEGF), a potent angiogenic and anti-apoptotic cytokine, had also successfully achieved improvement in erectile response in the rats. Besides increase in the endothelial and smooth muscle cells, transduction with VEGF gene might be advantageous as it could enhance the survival of injected MSC too.

Adipose-derived stem cells (ADSC) had also been used to treat impotent Zucker Diabetic Fatty obese type-II diabetic rats by Garcia and his team.[136] The autologous ADSC were injected into the proximal base of the penis and three weeks after treatment, they found that there was a significant increase in the average of mean intracorporal pressure per mean arterial pressure (ICP/MAP) during carvenous nerve stimulation when compared with the untreated group. Although only a small number of ADSC were detectable, the treatment group demonstrated an increase in neuronal nitric oxide synthase (nNOS) in the dorsal penile nerve, endothelial cell number and generally less cell death in the corpus carvenosum. Meanwhile, Albersen *et al* [137] injected the ADSC or its lysate into a rat model of cavernous nerve crush injury and found that both treatments resulted in significant recovery of erectile function, as compared with injured control group. The treatment groups showed a significantly higher nNOS and smooth muscle content, and less fibrosis compared with the injured controls. These observations imply that stem cells exert its regenerative properties by the release of intracellular preformed substances or by secretion of certain active factors which may be involved in proliferation of smooth muscle cells, neuron preservation and rescue of cell death.

Emerging use of mesenchymal stem cells in treating ocular diseases

Optimism for MSC use in ocular disorders is based on observations that MSC can differentiate into neurons *in vitro*[138,139] and specific retinal neurons *in vivo* following damages caused by chemical induction[140] or burn with laser.[141] Insertion of noggin gene by viral transduction into MSC alone could drive them to trans-differentiate into neuron and photoreceptor cells in culture.[142] The limited MSC integration into the retina and failure to show direct differentiation of MSC into any neural retinal cells in some animal studies[143-146] have somehow led to re-evaluation of the potential of MSC and its mechanisms for preservation of visual function.

Results from following studies have, however, supported the notion that MSC could excrete certain trophic factors beneficial to preserving visual function. This has been clearly demonstrated in a study carried out by Lund and his colleagues in 2007.[147] His team had injected MSC derived from different tissues into the subretinal layer of the Royal College of Surgeons rat model of retinitis pigmentosa (RP), in which most photoreceptors would degenerate as they age due to genetic defect. The findings showed that MSC derived from umbilical cord were able to sustain visual function of the rats, as assessed by electroretinogram, for three months despite limited retinal integration. The team also found that MSC released a number of neurotrophic factors including interleukin-6 (IL-6), which have been shown to be effective in rescuing photoreceptors in different retinal degeneration models following direct injection into the vitreous or via viral vector delivery. In another study, the scientists exposed green light to Sprague-Dawley (SD) rats to induce retinal light damage, which primarily caused photoreceptor apoptosis similar to RP. After 10 days, bone marrow MSC were injected into the subretinal layer. Examination at two weeks later showed no transplanted MSC was observed to differentiate into neural or retinal cell to replace the lost

photoreceptors. Similar to the previous paper, they showed positive expression of apoptosis protective cytokines such as ciliary neurotrophic factor (CNTF), basic fibroblast growth factor (bFGF) and brain-derived neurotrophic factor (BDNF) in the supernatant of MSC. However, they were only successful to locate the immunoreactivity of BDNF in the transplanted cells.[148] Interestingly, in the same year, Wang *et al* [149] reported that intravenous infusion of MSC had not merely successfully preserved apoptosis of photoreceptors and reduced the number of pathological vascular complexes in the RCS rats, but also demonstrated that MSC could migrate from the systemic circulation into the retina and perhaps more significantly, the up-regulation of the Müller cell-derived neuroprotectant CNTF.

Emerging application of mesenchymal stem cells in aesthetic medicine and surgery

MSC have also been used for reconstructive or cosmetic plastic surgery. In children suffering from craniofacial defects, repair of large skull defect can be difficult due to the limited amount of autogenous bone available. Alloplastic materials cannot be used in children because of continuous calvarial bone growth. As MSC can differentiate into osteogenic cells and possess osteo-inductive properties, the use of autologous MSC for bone regeneration becomes a new therapeutic option. Lendeckel[150] first described the use of autologous ADSC with fibrin glue to stimulate growth of milled cancellous bone and repair of the calvarial bone defects on a 7 year-old female following a fall. Postoperative healing of the patient was uneventful and clinical follow-up had shown new bone formation without neurological deficits.[151]

The other success of bone reconstruction using autologous MSC on a 65-year old man was reported by a Finnish team five years later. This man has undergone a hemimaxillectomy twenty eight months earlier due to large recurrent keratocyst. The scientists harvested stem cells from the fat tissue and culture expanded the ADSC until a sufficient number of cells were obtained for seeding onto a β-tricalcium phosphate scaffold moulded into the shape of the defect. The engineered tissue construct was implanted into the patient's rectus abdominis muscle, and resected eight months later for re-transplantation into the maxillofacial defect. The patient was able to regain full oral function and remained so at twelve months of follow-up.[152]

Fat grafting for cosmetic soft tissue augmentation, specifically on breast enhancement, has received much attention over the past few decades. The mature fat transferred as a graft usually has a low survival rate (25 to 60%) and undergoes atrophy, particularly due to the relative deficiency of tissue-specific progenitors in aspirated fat tissue and lack of blood supply.[153] Therefore, Yoshimura and his colleagues[154] developed a novel method to further concentrate the number of stem cells in a lipoaspirate fat, termed cell-assisted lipotransfer (CAL) for breast implants. In this technique, half of the lipoaspirated fat was centrifuged to isolate the stromal vascular fraction (SVF) which contains abundance of ADSC. The remaining lipoaspirate, also serves as biological scaffold for fat grafting, would then further enriched in its stem cells contents by the addition of SVF. This mixture was injected into the breasts of 15 female patients in Japan and the surviving fat volumes were recorded to range from 40 to 80% at 12 months later. Most patients developed natural breast softness without any palpable nodules and were satisfied with the texture, softness, contour and symmetry of their breasts. Moreover, no cyst formation or micro-calcifications were detected. Therefore, CAL provides a feasible approach for delivery of autologous ADSC and may be a good alternative to replace the use of artificial implants for breast enhancement as implantation of autologous ADSC is relatively safer.

MSC have also shown promising application in cosmetic dermatology, especially in the treatment of skin aging and pigmentation. In one study, ADSC or ADSC-conditioned medium (ADSC-CM) had been injected on the back of micropigs and histological evaluation showed a small, but not definite, increase of dermal thickness. Western blot analysis showed a distinctive increase in collagen expression in both injected skin samples. In their pilot program, intradermal injection of purified autologous processed lipoaspirate showed improvement of general skin texture, reduced wrinkle, and increased periorbital dermal thickness in a photo-aged patient. It was demonstrated that these cells could excrete certain proteins like fibroblast growth factor (bFGF), transforming growth factor (TGF)-β1, hepatocyte growth factor (HGF), keratinocyte growth factor (KGF), platelet-derived growth factor (PDGF)-AA, vascular endothelial growth factor (VEGF) and fibronectin that might overall contribute to enhance fibroblast migration and collagen

synthesis in the injected site.[155] In addition, TGF-β1 had also been identified to possess anti-whitening effect by increasing the rate of degradation of tyrosinase and tyrosinase-related protein 1 (TRP1), two key enzymes in melanin biosynthesis.[156]

Other than skin rejuvenation, MSC have been explored to treat hair loss. Androgenetic alopecia is a heritable, androgen- and age-dependent process which can cause progressive loss of hair follicles on the scalp.[157] It remains elusive whether decline in stem cell numbers or function of stem cell accounts for the failure to reproduce new hair follicles or changes in the microenvironment as a result of aging affect the stem cell activity.[158] Despite that, *in vitro* observations appear to support the use of conditioned-medium in the treatment of androgenetic alopecia or other forms of hair loss as it contains trophic factors to induce proliferation of human epidermal keratinocytes (HEK) and human follicle dermal papilla cells (DPC), and promote hair growth in the telogen-matched C3H/NeH mice.[159] MSC derived from the bone marrow and umbilical cord could form dermal papilla like tissues (DPLT) *in vitro* and generated new hair growth when injected subcutaneously in the nude mice.[160] Notably, dermal papilla cells isolated from the hair follicle possess characteristic resemblance to MSC.[161,162]

CLOSING THOUGHTS

Overall, the regenerative capacity of an injured tissue depends on the number of quiescent local progenitor stem cells, the microenvironment which the cells reside and their capability to proliferate and differentiate into specific cells upon receiving signals following injury. However, when local progenitor stem cells are overdriven to proliferate and differentiate in diseased states, this may lead to a depletion of stem cell population and loss of capability to repair effectively. A similar situation occurs in ageing tissues. This explains the general improvement of the well-being of old or damaged tissues when new stem cells are introduced at a targeted site in transplantation. Stem cells may act beyond their role in the production of cells through cell differentiation or rescue of cells through cell fusion, perhaps via the secretion of trophic factors. Can the trophic factors alone kick-start the local progenitors out of their quiescent state to do their job? If yes, conditioned-medium would abrogate the need for exogenous stem cells in some clinical cases not related to genetic defect, such as in inflammatory-related diseases or in the beauty industry. This approach will undoubtedly eliminate the risks of malignant transformation of MSC, unwanted mesenchymal lineages differentiation, and suboptimal targeted differentiation and unknown fate of MSC following transplantation.[163-167] On the other hand, conditioned medium is most likely not able to provide a desired and long lasting result, and may intuitively suggest the need for co-treatment with exogenous stem cells. Intervention such as microenvironment nourishment with conditioned medium prerequisite to stem cells transplantation might be useful to enhance the survivability of stem cells at already damaged tissue site. Or would multiple low dose injection of stem cells spread over a treatment course provide better results compared with single injection of stem cells in large amount, on the basis that the initial dose can prepare the microenvironment via its paracrine effects to fit the settlement of the next dose of stem cells? Indeed, the reparative mechanisms and the influence of microenvironment on stem cell activity need to be understood to achieve an effective stem cell-based therapy.

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