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CHAPTER FIVE

Current trends and promising clinical utility of IPSC-derived MSC (iMSC)

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Contents

Abstract

Mesenchymal stem cells (MSCs) differentiated from human induced pluripotent stem cells (iPSC) or induced MSC (iMSCs) are expected to address issues of scalability and safety as well as the difficulty in producing homogenous clinical grade MSCs as demonstrated by the promising outcomes from preclinical and clinical trials, currently ongoing. The assessment of iMSCs based in vitro and in vivo studies have thus far showed more superior performance as compared to that of the primary or native human MSCs, in terms of cell proliferation, expansion capacity, immunomodulation properties as well as the influence of paracrine signaling and exosomal influence in

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cell-cell interaction. In this chapter, an overview of current well-established methods in generating a sustainable source of iMSCs involving well defined culture media is discussed followed by the properties of iMSC as compared to that of MSC and its promising prospects for continuous development into potential clinical grade applications.

1. Introduction

Despite the numerous established studies and their promising outcomes of mesenchymal stem cell (MSC) in regenerative medicine, disease modeling and cancer therapy, there exist major hurdles in bringing MSC therapy into mainstream clinical applications owing to the difficulty of acquiring sufficient number of such cells from single donor, unsustainable cell expansion and the heterogenous population of extracted cell sources.[1] These technical challengers have led to observations of inconsistent results on the proliferation capacity, immunomodulation properties and therapeutic potential of MSCs. However, recent developments in induced MSCs (iMSC) which are basically MSC-like cells differentiated from iPSCs are expected to overcome the issue of scalability and safety as well as the difficulty in producing homogenous clinical grade MSCs as demonstrated by the promising outcomes from preclinical and clinical trials, currently ongoing.[2,3]

2. Generation of iMSCs from iPSC

Technology advancement over the past decade in iPSCs have provided reliable methods on derivation of iMSCs for therapeutic and regenerative medicine[4,5] Since, iPSCs can be generated by using nonviral methods and without the use of c-myc,[6] differentiated iMSCs are relatively safe for clinical applications. Compelling evidence have shown that iPSCs are inducible towards mesenchymal like cells (iMSCs) with similar genotypic and phenotypic signatures as that of the primary MSCs, as well as stable across prolonged passage without chromosome abnormalities.[7,8] Similarly, MSC-like cells can be differentiated from iPSCs by both direct and indirect differentiation methods. These methods were further classified into detailed approaches as such induction using growth factors, small molecule inhibitors, physiochemical stimulation from biomaterial matrices and forced ectopic expressions (Fig. 1).[9]

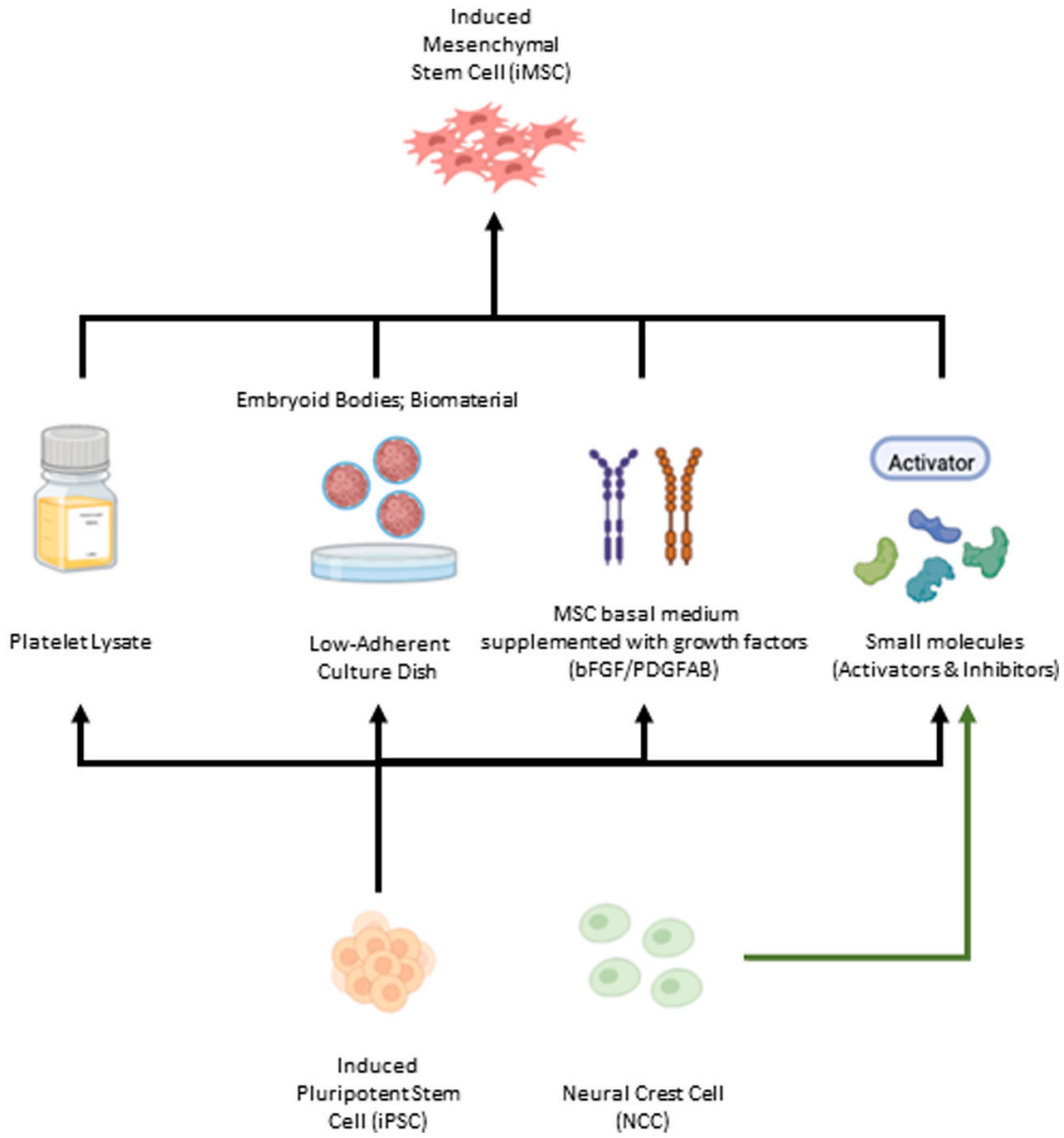

Fig. 1 Methods of iPSC differentiation to MSC. *Adapted from Aldoghachi et al.*[9]

Traditional differentiation protocol involved one step method by switching iPSCs medium into MSCs medium. MSC basal medium is supplemented with growth factors namely platelet-derived growth factor alpha polypeptide b (PDGF-AB) or bFGF which increases the formation of fibroblast-like cells.[10] On the other hand, another classical way to establish iMSCs is by induction of spontaneous differentiation from iPSCs into embryoid bodies (EBs).[11] EBs are 3D cell aggregates comprising three germ layers (ectoderm, endoderm and mesoderm) derived by using fetal bovine serum (FBS) supplemented medium. Generally, the use of FBS or albumin products potentially depletes pluripotency and enhance the differentiation efficiencies in iPSCs. EBs then further directed into MSC-like cells using MSCs medium.[12]

However, over the years, iMSCs differentiation protocol were improvised by introducing various kinds of small molecule biochemicals into basal culture medium. Addition of Wnt signaling activator and Activin/Nodal/TGFβ signaling inhibitor in iMSCs induction medium decreases pluripotent signals (OCT3/4, SOX2, NANOG) subsequently gives rise to MSC-like cells.[10] Besides, physiochemical simulation from biomaterial matrices was shown to induce iMSC differentiation from iPSCs alongside with MSC specific growth factors. The use of coating matrices such as fibronectin, collagen and gelatine are well established to date.[10]

Forced ectopic expression of HOX genes were demonstrated as another method of generating iMSCs. HOX genes were found highly expressed in primary MSCs includes vascular wall (VM), bone marrow (BM) and adipose tissues (AT). In fact, a study by Steens et al. showed lentiviral vector expressing a subset of vascular wall specific MSC HOX genes successfully differentiated iPSCs into VM-iMSCs.[8]

3. General characteristics of iMSC in comparison to primary MSC

As a common practice, when an MSC-like cell is differentiated from iPSC, the verification of its MSC properties is based on the International Society for Cell and Gene Therapy (ISCT) minimum criteria for defining mesenchymal stem/stromal cells (MSCs) based on the morphology, expression of MSC surface markers, and specific mesodermal lineage differentiation.[13] Generally, the defining features include cells demonstrating fibroblast-like structures and adherence to plastic, positively expressing MSC markers (CD90, CD73, CD44, and CD105) and negatively expressing hematopoietic markers (CD11, CD45, CD34, CD14, CD19, HLA-DR), as well as the ability to undergo trilineage differentiation into adipocytes, chondrocytes, and osteocytes.[13] These properties are basically reflected in previous iMSC related studies[7,14–20] (Fig. 2) and a comparison of the various iPSC source used in the generation of iMSC as well as the trilineage differentiation capacity are summarized in Table 1.

Based on the general characterization of iMSC compared to that of their native or primary MSCs, certain unique features are apparent in iMSCs. In a study involving iMSC generated from skin fibroblast of a mouse model, CD90 was not express but CD160 (exclusively express for MSC but not in skin fibroblast) was positive for both iMSC and the

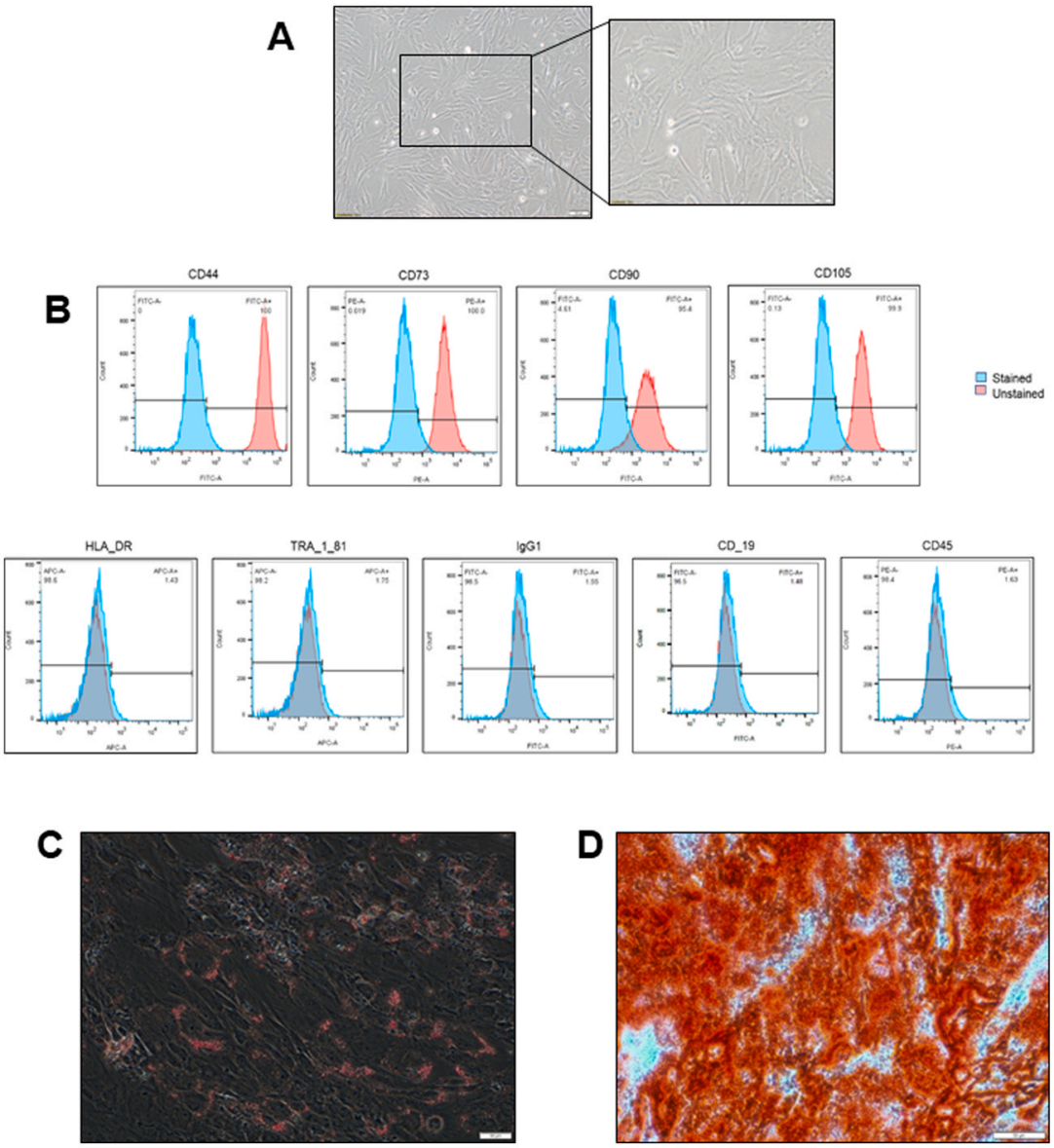

Fig. 2 Characterization of induced mesenchymal stem cells (iMSCs). *Adapted from Loh et al.*[7]

primary MSC counterpart.[22] Another observation was that although many of the previous studies reported the ability of iMSC to differentiation into all three lineages, when compared to that of their respective primary MSCs, some studies showed either lower levels of adipogenic differentiation[19,23,24,28] or lower adipogenic and chondrogenic differentiation.[27] In addition, significantly longer telomere lengths were previously reported in iMSCs,[18,21,23] and a higher proliferation rate as well as the ability to continue to proliferate under prolonged passaged was notably evident as compared to that of their primary parental MSC cells,.[18,20,23,29] Similarly, chromosomal abnormalities in iMSC expansion were not detected even up to passage 15.[26]

Table 1 Comparison of Trilineage differentiation potential and growth characteristics of iMSC with native or primary MSC.

Authors/ Reference	Source of IPSC derived MSC (iMSC)	Primary or Native MSC used for comparison	Trilineage differentiation capacity/potential of iMSC	Specific characteristics of iMSC
Umrath et al.[21]	Human jaw periosteal cells (JPCs) via transfection of a self-replicating RNA (srRNA)	Parental primary JPCs	All three lineage differentiation were clearly observed	• iMSCs showed significantly longer telomere lengths. • Proliferation, mitochondrial activity, and senescence-associated beta-galactosidase activity indicated early senescence of iMSCs.
Soontararak et al.[22]	Transgene integration-free iPS cells were generated from mouse skin fibroblasts	Abdominal and inguinal adipose tissues of 10-weeks old female CD-1 mice	All three lineage differentiation were clearly observed	CD90 was negative for iMSC but both MSC And iMSC were positive for CD160 (exclusive for MSC but not for skin fibroblast).
Wei et al.[18]	Human dermal fibroblasts using OCT-4, SOX-2, KLF-4, c-Myc via retroviral-based reprogramming	Bone marrow and umbilical cord blood-derived MSCs	All three lineage differentiation were clearly observed	• Cell proliferation was more than 32 population doublings without cellular senescence. • Showed superior pro-angiogenic and wound healing properties.

Zhou et al.[20]	BM-MSC of osteonecrosis of the femoral head (ONFH) patient using OCT-4, SOX-2, KLF-4, c-Myc via retroviral-based reprogramming (ONFH-BMSCs)	Normal BM-MSC	Osteogenic and adipogenic differentiation was clearly observed	• DNA methylation patterns of iPSC-MSCs were similar to those of normal BMSCs. • The proliferation of iPSC-MSCs was higher and no tumorigenic ability was exhibited. • iPSC-MSCs was equivalent to normal BMSCs in preventing bone loss and promoting bone repair in the necrosis region of the femoral head.
Hynes et al.[14]	Tail-tip fibroblasts from NOD/Lt mice using retroviral transduction of the coding sequences of three transcription factors, OCT4, SOX2, and KLF4, in combination with a histone deacetylase inhibitor, valproic acid.	NA	All three lineage differentiation were clearly observed	Control acute and chronic inflammatory responses associated with the destruction of periodontal tissue.

(*continued*)

Table 1 Comparison of Trilineage differentiation potential and growth characteristics of iMSC with native or primary MSC. (*cont'd*)

Authors/ Reference	Source of IPSC derived MSC (iMSC)	Primary or Native MSC used for comparison	Trilineage differentiation capacity/potential of iMSC	Specific characteristics of iMSC
Lee et al.[23]	Primary BM-MSC were reprogrammed via Sendai viral vectors encoding Oct4, Sox2, Klf4 and c-Myc	Autologous BM-MSC (donor)	• Three different iMSCs exhibited variable changes in osteogenic differentiation potential • All of the iMSC lines exhibited lower levels of adipogenic differentiation potential than parental MSCs	• Higher proliferative potential than their parental MSCs over 15–17 passages. • Longer telomere lengths. • Variations in differentiation potential in a donor cell-dependent manner. • More potent immune suppression of allogenic immune responses.
Jakob et al. (2021)	Human mucosa mesenchymal stem cells (mMSCs) of the upper respiratory tract were reprogrammed into iPSCs by using the plasmid-based integration-free reprogramming method	mMSCs	mMSCs and iP-MSCs show similar cell characteristics in terms of morphology, clonogenic potential, differentiation, and surface phenotype	iP-MSCs demonstrated related immunosuppressive capacity as mMSCs including the secretion of cytokines, and T cell inhibition.
Hynes et al.[15]	Human foreskin–derived iPSC	NA	All three lineage differentiation were clearly observed	iPSC-MSC like cells significantly increased the amount of regeneration and newly formed mineralized tissue present.

Zhao et al.[19]	Male blood cell-derived human iPSC line CY2 was generated with episomal plasmids	BM-MSC	Osteoblasts or chondrocytes after corresponding differentiation was significantly higher, whereas the up-regulation of most adipogenic markers was significantly lower compared with BM-MSCs	Tumor-tropic but have much less potential to promote tumors than bone marrow MSCs
Kang et al.[24]	Normal human dermal fibroblasts (NHDFs) via Lentivirus-mediated reprogramming	BM-MSC	Adequate osteogenicity and chondrogenicity but less adipogenicity	-
Diederichs and Tuan[25]	Bone marrow stromal Cells (BMSCs) were reprogrammed to obtain M-iPSCs via lentiviral overexpression of the four well-described pluripotency factors Oct3/4, Sox2, Klf4, and c-Myc	Parental BMSCs	Capable of differentiation in vitro along the osteogenic, chondrogenic, and adipogenic lineages.	iMPCs displayed a unique expression pattern of mesenchymal and pluripotency genes and were less responsive to traditional BMSC differentiation protocols.

(*continued*)

Table 1 Comparison of Trilineage differentiation potential and growth characteristics of iMSC with native or primary MSC. (*cont'd*)

Authors/ Reference	Source of IPSC derived MSC (iMSC)	Primary or Native MSC used for comparison	Trilineage differentiation capacity/potential of iMSC	Specific characteristics of iMSC
Rajasingh et al.[26]	Non-viral, mRNA-based reprogramming of human Urinary epithelial (UE) cell	Human UC-MSCs	Capable of differentiating into osteocytes, chondrocytes and adipocytes.	No chromosomal abnormalities even at later passages (P15). Wound-healing property demonstrated through migration assay was superior in iMSCs when compared to the UC-MSCs.
Xu, Shaw, Murphy, and Barry[27]	Retroviral reprogramming method (iPSCs were provided in the study)	BM-MSC	Weak adipogenic and and chondrogenic differentiation; similarly strong osteogenic expression	_

Devito (2019)	The native Wharton Jelly MSCs (WJ-MSCs) from two donors, were reprogrammed using CytoTune®-iPS Sendai Reprogramming kit	WJ-MSCs	Cells generated from iMSC using the TEX protocol had significantly higher expression of adipogenic and chondrogenic markers than those using ARG.	The ARG protocol resulted in iMSCs with a strong immunomodulatory potential and lower plasticity and proliferation rate, whereas the TEX protocol raised iMSCs with a higher proliferation rate, better differentiation potential, though weak immunomodulatory response.
Luo et al.[28]	Lentivirus-based reprogramming (OSKM) with human dermal fibroblasts	BM MSC	Similar osteogenic and chondrogenic capacities but less adipogenic potential.	Extracellular matrix structure and organization were significantly different between the iMSCs and BM-MSCs

Aside from the general characteristics of iMSC, Luo et al. compared transcriptome level data between iMSCs to bone marrow-derived MSCs (BM-MSC) and noted that in particular, the extracellular matrix structure and organization were significantly different between the two sources of MSCs.[28] Likewise, a study which compared iMSC with BM-MSC from the same donor demonstrated an array of unique expression pattern of mesenchymal and pluripotency genes from iMSCs and these cells were also less responsive to traditional BM-MSC differentiation processes[25] with variations in differentiation capacity in a donor cell-dependent manner.[23] Taken together, these findings strongly indicates that iMSCs have their own distinct characteristics and for these cells to be applicable in clinical application, their iPSC source as well as the method of iPSC generation may be of more immediate relevance rather than to fully comply with the ISCT criteria.[5,23,25]

4. Properties of iMSC that may cater to large scale production of homogenous clinical grade MSCs

4.1 Promising immunomodulatory properties

Numerous studies done on iMSC have demonstrated its strong immunomodulatory properties and one study showed such property to be independent of cell-of-origin or reprogramming system used along with immunomodulation toward CD4 and CD8 cells.[30] Furthermore, comparative studies between iMSCs and their native MSCs have shown more superior wound healing properties seen from migration assays[26] and pro-angiogenic properties[18] as well as more potent immune suppressive effects on allogenic immune responses.[23]

4.2 Non-tumorigenic

Despite the known risk of tumor properties acquired from cell reprogramming into iPSCs, downregulation of c-Myc was evident in iMSCs[30] and overexpression of bone regeneration protein, BP6 from a genetically modified iMSC did not show any evidence regarding nonviral overexpression of BMP6 inducing tumorigenesis.[31] Interestingly, by comparing with BM-MSCs with coculture studies, iMSCs were shown to reduce the promotion of cancer properties of cancer cells with significantly lower expression of genes related to epithelial–mesenchymal transition, invasion, stemness, and growth of cancer cells. These results were attributed to the

low expression of IL-1, TGF-beta, and TSG6 in iMSCs compared to that of BM-MSC.[19] Moreover, it was also shown to possess tumor-tropic ability by migrating to tumor sites based on in vitro and in vivo assays involving human cancer xenograft models of colorectal cancer cells and MDA-MB-231 breast cancer cells.[19] Our results have also shown that iMSC suppresses the growth of lung cancer cell line, H1975 by releasing proapoptotic cytokines into coculture media.[7]

4.3 Resetting of age-related DNA methylation and a more homogenous cell population

There has been compelling evidence of age-related DNA methylation in iMSCs, accumulated during in vitro culture, were being reset as demonstrated by the rejuvenation signature and reduced heterogeneity in iMSC clones.[11,32,33] These epigenetic rejuvenation lead to stronger regeneration ability of iMSCs in animal disease models and higher proliferation rates when compared to primary MSC.[19,26,34] Therefore, despite maintaining senescence-related changes as do primary MSCs,[35] cellular aging may be better managed through expanding iPSCs in the pluripotent state which acts as a rich source of iMSCs which in turn could now be generated at low passage number; allowing its further large scale expansion for cell-based therapy. Moreover, clonal dominance[36] was observed during culture expansion of iMSCs which lead to more homogenous cell population and this property was attested by Bloor et al. and Ozay et al. in their preclinical and clinical trial studies respectively involving the use of iMSC in Graft-vs-host disease.[2,3]

5. Applications of iMSC

5.1 iMSC in regenerative medicine

Several animal studies using iMSCs in replacement of MSCs have shown significant recovery on tissue regeneration and tissue damage repair. Hynes and colleagues implanted iMSCs into rat periodontal defect model and showed that iMSCs can promote periodontal regeneration and formation of new mineralized tissue.[15] In another study, iMSCs-induced osteoblast transplantation into a calvarial defects area of mice showed a significant recovery of bone formation.[37] Moreover, a genetically modified iPS-derived MSCs overexpressing bone morphogenetic protein 6 (*BMP6*) can regenerate nonunion bone defects in mice more efficiently than that of

human BM-MSCs. The authors strategically used *BMP6* as it is known to regenerate bone defects and induce bone formation in vivo without the need for massive quantities of *BMP* protein or harvested bone grafts.[31]

The use of iMSCs was also demonstrated in angiogenesis and repair of cardiac wound. Meow and his team implanted iMSCs and BM-MSCs into the ischemic site in mouse hindlimb, the effects of iMSCs shows significantly reduced in the ischemic damage when compared to BM-MSCs.[17] The assessment of iMSCs with BM-MSCs and UC-MSCs repair ability in myocardial infarction and dysfunction mouse model has shown that iMSCs have more significant ability in cardiac wound recovery and proangiogenic potency.[38–40]

For the use of skin regeneration, Kim and colleague compared the effects of extracellular vesicles (EVs) from iMSC and UC-MSCs. Both EV sources were shown to improve collagen and fibronectin secretion and expression that lead to improved cell proliferation in human keratinocyte and dermal fibroblasts that hasten the regeneration time. However, treatment using iMSC shows a higher increase in proliferation and fibronectin release in human keratinocytes when compared to that of MSC-EVs. The application of iMSC-EVs directly on the wound sites in the rat model have shown a higher effectiveness in reducing the scar widths, increasing re-epithelization and promoting collagen deposition.[41] In the study using iMSC-EVs in vascular disease through in vivo and in vitro model, the effects were promising and beneficial. The use of iMSC-EVs was shown to activated angiogenesis-related molecule expression, proliferation of endothelial cells, promote tube formation, increase collagen deposition, increase micro vessel density, reduce brain tissue loss, improve cell migration and improving disease condition.[16,28,42–44] Furthermore, the high proliferation and expansion rate of iMSCs has allowed a more large scale collection of iMSC-EVs as compared to MSC-EVs allowing the future use of iMSC-EVs in a clinical setting. Further reviews on exosomes and EVs from iMSC for regenerative medicine have been comprehensively described in Aldoghachi et al. and Bertolini et al.[9,45]

5.2 iMSC in cell-based cancer therapy

There have been studies with genetically engineered iMSC revealing promising therapeutic outcomes on several cancer types.[16,46,47] One study involving iMSCs expressing Interleukin 24 (IL24-iMSCs) induced in vitro apoptosis in B16-F10 melanoma cells more efficiently than control iMSCs when co-cultured in Transwell assays. Further follow up analysis using in

vivo tumor xenograft studies in mice had also demonstrated that IL24-iMSCs inhibited melanoma growth more than that of the control iMSCs. Substantial evidence based on immunofluorescence and histochemical analysis showed larger necrotic areas and cell nuclear aggregation in tumors treated with IL24-iMSCs than control iMSCs, indicating that IL24-iMSCs inhibited tumor growth by inducing apoptosis.[16]

Another genetically modified iMSC demonstrated the incorporation of tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) in iMSC (iMSC-TRAIL) via site-specific integration into ribosomal DNA induced apoptosis in melanoma, liver, breast, and lung cancers in vitro and inhibited tumor growth through the activation of apoptotic pathways in vivo.[47] Based on iPSC-Derived Hereditary Breast Cancer Model, Portier et al. generated two patient-specific iMSCs from peripheral blood mononuclear cells (PBMC) carrying the BRCA1 mutations [iMSC-BRCA1(-)] and when compared to the iMSC generated from normal controls, iMSC-BRCA1(-) exhibited pro-angiogenic properties as high expression of angiogenic factors namely VEGF, PDGF, ANGPT, and HIF-1 were observed. iMSC-BRCA1(-) was also found to demonstrate higher capacity in generating tube-like structures and vessels based on in vitro and in vivo studies compared to that of normal controls.[46] In addition, the treatment of metastatic prostate cancer showed positive effects with the use of extracellular vesicles (EVs) derived from iMSCs.[48]

5.3 iMSC in stem cell therapy

The use of MSCs derived from tissue sources for therapeutic applications is limited by their restricted expansion capacity and donor-to-donor variability. To overcome these challenges, MSCs derived from iPSCs have been developed, as they demonstrate greater expandability and offer potential for tissue repair therapies. The use of iMSCs has emerged as an innovative choice in regenerative medicine, as they have several significant advantages, including unlimited growth and stable cell quality. Furthermore, iMSCs have been successfully applied to the treatment of various diseases.[3] The application of MSCs as a therapy for early-stage osteonecrosis of the femoral head (ONFH) shows promising results but not with limitations such as restricted cell growth and donor availability. To overcome these issues, researchers have explored using iMSCs. These cells have similar properties to normal MSCs, but with higher cell growth and no cancer risk. Studies using rat models of ONFH showed iMSCs are just as effective as normal MSCs in preventing bone loss and promoting bone repair.

Transplanting iMSCs also helps with bone repair and blood vessel growth in the ONFH region.[20] Moreover, Chen et al. have demonstrated that iMSC therapy can reduce brain infarct volume and improve neurological function in rats with intracerebral hemorrhage. The therapy works by reducing inflammation and oxidative stress.[49] In addition, iMSCs utilize the hyaluronic acid-CD44 interaction to promote epithelial cell proliferation in an Akt-dependent manner through TSG6, accelerating mucosal healing in a mouse colitis model, demonstrating a patient-specific "off-the-shelf" form of therapy for IBD (inflammatory bowel disease).[50] In a similar study mouse IBD model, microbiome normalization was demonstrated to correlate with the intestinal healing effects.[22]

In addition to basic research, iMSC have already been commercialized and are available on the market, such as Cymerus. Cymerus is a technology by Australian company Cynata Therapeutics that uses iPSCs to create a consistent and scalable supply of MSCs. These MSCs can be used to treat various medical conditions, including autoimmune, respiratory, and cardiovascular diseases. Cymerus overcomes many limitations of traditional MSC production methods and has the potential to advance regenerative medicine. Thavapalachandran et al. investigated the regenerative potential of induced pluripotent stem cell-derived iMSCs from the Cymerus MSC population in a rat model of myocardial ischemia-reperfusion (I/R).[51] In the rodent model of myocardial I/R, intramyocardial administration of iMSCs (Cymerus MSCs) provided superior therapeutic efficacy compared to conventional BM-MSCs. Due to its manufacturing scalability, iMSC therapy provides an exciting opportunity for an "off-the-shelf" stem cell therapy involving cardiac repair.[51] Organ transplantation surgery can save lives, but long-term survival is challenging due to rejection reactions and toxic side effects. In preclinical studies, Cymerus iMSCs showed potential to improve graft survival and oxygenation, and to prevent airway damage and collagen accumulation creating a feasible treatment option for transplant recipients.[52]

Interestingly, MSCs can transfer mitochondria to damaged cells, effectively providing functional mitochondria to the damaged cells and preventing cell degeneration caused by mitochondrial damage. Previous study investigated whether mitochondrial donation from iMSCs could sustain retinal ganglion cell (RGC) survival and restore retinal function.[53] The results showed that injection of iMSCs increased the survival rate of RGCs with improved retinal function. GFP-labeled human mitochondria from iMSCs were detected in RGCs of mouse retina, and only human

mitochondrial DNA was detected in mouse retina after iMSC treatment.[53] In addition, MSCs is known to transfer mitochondria to damaged heart cells, but its mechanism of transfer is not well established. However, Zhang et al. demonstrated that iMSCs have higher mitochondrial transfer efficiency than BM-MSCs due to high expression of intrinsic Rho GTPase 1 (MIRO1).[54] iMSCs also responded more readily to tumor necrosis factor α (TNF-α)-induced tunneling nanotube (TNT) formation to transfer mitochondria to heart cells, which is mediated through the TNF-α/NF-κB/TNFαIP2 signaling pathway. Therefore, transplanting iMSCs into a mouse model of heart damage caused by chemotherapy resulted in better mitochondrial retention and preservation compared to BM-MSCs.[54] These studies conclude that intravitreal transplantation of iMSCs can effectively provide functional mitochondria to damaged cells and prevent mitochondrial dysfunction-induced cell degeneration.

6. Scaling up

MSCs are multipotent stromal cells derived from adult tissue[13] and when used for therapy, these cells demonstrated advantages over other methods, as they constitute a stem cell niche, support the growth of tissue-specific stem cells, and promote tissue regeneration.[55,56] Notably, MSCs exhibit low immunogenicity, and the use of allogeneic MSCs is typically safe.[57] Despite their excellent therapeutic efficacy, tissue-derived MSCs have several limitations, such as their limited proliferative potential, difficulties in standardization, loss of differentiation ability, and decreased therapeutic efficacy during expansion.[58] However, single-cell-derived MSCs generated from iPSCs can overcome these limitations. MSCs can be bioengineered from iPSCs and have the potential for efficient expansion, easy standardization, and unlimited differentiation from iPSCs. Here, we will discuss the techniques for scaling up MSCs and the potential applications of iMSCs.

There are two distinct methods available for amplifying MSCs using cultivation techniques: the 2D cultivation technique and the 3D stirred-tank and other dynamic bioreactors. In 2D single-layer conditions, undifferentiated MSCs are maintained and expanded at low densities in culture containers with flat surfaces, with cells adhering to the plastic surface of culture plates or flasks.[59] To increase the number of cells under 2D conditions, multi-layer flasks or cell stackers can be employed to expand the surface area of the culture vessel.[60] Small, medium, and large-

scale cell manufacturing in flat 2D static cultures can be achieved through tissue culture flasks up to 10–40 layers stack systems, with various commercialized cell stackers are available. This manufacturing method is commonly referred to as "horizontal expansion," wherein the expansion unit size remains constant, and parallel units are multiplied. However, the limitations of flat culture systems include the need for larger surface areas for clinical-scale cell growth, the handling of large volumes of liquid during medium changes, passaging, and cell harvesting, as well as the need for large-scale incubators, which occupy considerable space in cleanrooms.[61,62] Moreover, this technology can lead to a restricted surface-to-volume ratio, resulting in a bottleneck during the manufacturing process. Additionally, the environment inside the battery stacker is heterogeneous, with each flask comprising a unique microenvironment that is vulnerable to contamination, batch-to-batch variability, and non-uniform surface treatment between suppliers.[63] Furthermore, due to the open nature of cultivation and the need for numerous cell passages to generate sufficient cells for research purposes, the process is labor-intensive and susceptible to contamination. Typically, 2D culture conditions are static and lack the ability to monitor and control culture conditions through sensors, which is not optimal for cell manufacturing.[64]

The bioreactor system of stirred tank and other dynamic reactors can be easily operated and can expand cell culture volume through computer-controlled online monitoring devices that can control process variables such as pH, temperature, dissolved oxygen, and carbon dioxide concentration.[65] Although, stirred-tank bioreactors also introduce an important complication: fluid mechanics.[66] A typical stirred-tank bioreactor is usually a cylindrical vessel with impellers that provide constant motion and is widely used as an amplification bioreactor system for MSC-based cell therapy, especially for homologous and heterogeneous cell therapy that requires the production of large amounts of cells. The stirred-tank configuration can achieve effective mixing, but non-uniform flow may occur under certain conditions or regions in the bioreactor where turbulence can occur.[66] Bioreactor amplification technology promotes dynamic suspension cultivation, which is quite different from static two-dimensional cultivation. Cells in the bioreactor can be expanded as suspension cell aggregates or seeded onto small solid spheres called microcarriers. For MSC, it has been found that using this method for expansion can preserve a stable phenotype,[67] at least when considering the minimal definition of MSC. As self-assembling cell aggregates or spheroids can simulate in situ

conditions, cell morphology can better represent body tissues.[68] The medium for forming cell aggregates into spheres includes adhesion molecules that promote cell-to-cell attachment, including laminin, integrin, E-cadherin, and vitronectin.[69] However, for GMP production, these recombinant human proteins are prohibitively expensive, and microcarrier-based cultivation systems are particularly suitable for MSC expansion. Compared to 2D systems, microcarrier beads have a larger surface area, which can maximize MSC attachment. Bioreactors that use microcarriers can also operate at higher densities, thereby reducing supply or cost of goods (COG). Although the use of microcarriers and stirred tank systems for three-dimensional (3D) expansion manufacturing systems offer advantages, they also pose potential challenges. Further improvements tailored to the expansion of MSCs in dynamic culture systems are necessary to achieve invariant and reproducible MSC production for biological research and ultimate clinical applications. Furthermore, research is still required to gain a broader and more comprehensive understanding of the relationship between manufacturing methods and clinical efficacy, as well as how to optimize manufacturing for optimal clinical outcomes. This is particularly relevant to MSCs, which are applicable to a wide range of disease indications, and which may require different characteristics that can be customized using optimized manufacturing tailored to the disease.

7. Conclusion

In view of major concerns in primary MSC applications due to donor-based variability, limited sources, and unsustainable proliferation, iMSC on the other hand, have shown to be a promising alternative stem cell source in overcoming heterogeneity and scalable production of clinical grade MSCs. As iMSCs are derived from iPSCs, safety concerns have been addressed with the use of nonviral, non-integrative approach in producing iPSCs from donor cell source even with minimal starting material. The differentiated iMSC from these iPSCs are expected to be expanded with more homogenous cell populations and at a lower passaged number starting point ensuring better clinical grade cell types for various stem cell therapy.

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