hiPSC-derived iMSCs: NextGen MSCs as an advanced therapeutically active cell resource for regenerative medicine

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Abstract

Mesenchymal stem cells (MSCs) are being assessed for ameliorating the severity of graft-versus-host disease, autoimmune conditions, musculoskeletal injuries and cardiovascular diseases. While most of these clinical therapeutic applications require substantial cell quantities, the number of MSCs that can be obtained initially from a single donor remains limited. The utility of MSCs derived from human-induced pluripotent stem cells (hiPSCs) has been shown in recent pre-clinical studies. Since adult MSCs have limited capability regarding proliferation, the quantum of bioactive factor secretion and immunomodulation ability may be constrained. Hence, the alternate source of MSCs is being considered to replace the commonly used adult tissue-derived MSCs. The MSCs have been obtained from various adult and foetal tissues. The hiPSC-derived MSCs (iMSCs) are transpiring as an attractive source of MSCs because during reprogramming process, cells undergo rejuvination, exhibiting better cellular vitality such as survival, proliferation and differentiations potentials. The autologous iMSCs could be considered as an inexhaustible source of MSCs that could be used to meet the unmet clinical needs. Human-induced PSC-derived MSCs are reported to be superior when compared to the adult MSCs regarding cell proliferation, immunomodulation, cytokines profiles, microenvironment modulating exosomes and bioactive paracrine factors secretion. Strategies such as derivation and propagation of iMSCs in chemically defined culture conditions and use of footprint-free safer reprogramming strategies have contributed towards the development of clinically relevant cell types. In this review, the role of iPSC-derived mesenchymal stromal cells (iMSCs) as an alternate source of therapeutically active MSCs has been described. Additionally, we also describe the role of iMSCs in regenerative medical applications, the necessary strategies, and the regulatory policies that have to be enforced to render iMSC's effectiveness in translational medicine.

Keywords: mesenchymal stem cells ● MSCs ● induced pluripotent stem cells ● hiPSCs ● iMSCs

Introduction

Mesenchymal stromal cells (MSCs) are assorted cell preparations and only a rare subpopulation often referred to as 'mesenchymal stem cells' retains clonogenic proliferation ability & multilineage differentiation potential [1]. Mesenchymal stem cell preparations are significantly affected by starting cell source/material, such as bone marrow (BM), adipose tissue (AT) or other adult/perinatal tissue source; cell culture surface, media composition and other *in vitro* tissue culture conditions [1, 2, 9]. Furthermore, they acquire phenotypic, biochemical, molecular as well as functional changes during long-term *in vitro* culture expansion ending in replicative senescence [7, 8]. So far, MSCs are occasionally defined by their plastic adherent growth displaying fibroblast-like cellular colonies, a panel of

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positive (CD73, CD90, CD105) and negative cell surface markers (CD11b/CD14, CD34, CD45, CD79α/CD19) for phenotypic characterization and their capacity to differentiate towards at least trilineage differentiations such as adipogenic, osteogenic and chondrogenic lineages [2, 4]. Many researchers indicate that plasticity and immunomodulatory capabilities of the MSCs contribute towards unique therapeutic potentials of the MSCs [1, 2]. The bone marrow MSCs (BMMSCs) are considered to be the gold standard in the field of MSCs. However, their invasive accessibility and lower proliferation potential significantly undermine their ability to be considered for mainstream therapeutic applications [3]. The therapeutic potency of MSCs is often limited because of age or pathologically related impairments regarding cell survival, proliferation and differentiations potential of BMMSCs [4–6]. Before adult MSCs can exert its therapeutic potential *in vivo*; we must determine reasons behind their limited proliferation capability, quick down-gradation of their differentiation potentials and secrete minimal protective factors during their expansion *ex vivo*? [7, 8] The adult MSCs unveil time-limited functions under both *in vivo* and *in vitro* conditions [9, 10]. Exploration for an alternate source of MSCs resulted in several groups reporting successful isolation of MSCs like cells from foetal, neonatal [11–15] and embryonic stem cells (ESCs) [16–18]. As a result of the current deficit in adult MSCs regarding inadequacy in MSCs passages, cell numbers and consistencies in cellular behaviour; alternative, easily accessible, safe and healthy populations of MSCs are being considered for clinical applications [3]. The iPSC-derived MSCs (iMSCs) are emerging as an attractive option for obtaining a substantial population of stem cells in a sustained manner for regenerative medical applications [3]. The achievement of cell-based therapy of MSCs in preclinical trials has precipitated success in human translational applications [19].

Therapeutically active MSCs derived from human bone marrow

In the field of regenerative medicine, human mesenchymal stem cells (hMSCs) have transpired to be a promising candidate. Bone marrow-derived MSCs (BMMSCs) have been used as a predominant source of MSCs. Bone marrow-derived MSCs have been successfully used in a significant number of clinical and pre-clinical applications [20, 21]. In the early 20th century, Maximow and Friedenstein were the first to investigate the role of bone marrow fibroblast-like subset cells in maintaining the haematopoiesis [22]. The BMMSCs were first isolated and propagated under *in vitro* culture conditions in 1970s by Friedenstein *et al.* [21, 22]. In 1991, Arnold Caplan termed the cells MSCs based on the ability of the cells to give rise to distinct tissue lineages [23]. Maureen Owen further characterized the MSCs and observed the heterogeneity in its population [21, 24]. The *in vivo* administration of the MSCs in animal and humans has shown to be safe without triggering adverse immune reaction or any tumour formation [25]. Subsequently, MSCs have been shown to modulate the immune response and prevent graft-versus-host disease (GVHD) [26, 27]. Above all MSCs has been demonstrated to be effective in both pre-clinical and clinical stages in orthopaedic applications, cardiovascular

therapies, burns, wounds, ulcers, neurodegenerative disorders, spinal cord injury, autoimmune disorders, *etc.*, [28]. Mesenchymal stem cells exert their biological functions through cellular migration, local engraftment, self-renewal, plasticity, and secretion of various bioactive compounds. These intrinsic characteristics render the MSCs ideal for regenerative medical applications [29]. Moreover, MSCs can be engineered to secrete various bioactive factors through viral or non-viral-based methods, which enhance the capabilities of MSCs in therapeutic applications [30, 31]. The low proliferation potential of the adult BMMSCs renders the BMMSCs unsuitable for clinical applications [32]. Further, the limited accessibility, difficulty in obtaining patient consent and invasive procedure contribute towards un-usability of BMMSCs for routine clinical treatment. During specific times extraction of autologous MSCs from the patients might be counter-productive for the management of the patients [3]. The BMMSCs are short-lived and hence cannot ensure consistent, long-lasting immune regulatory functions both *in vivo* and *in vitro* [33]. Moreover, the adult MSCs undergoes replicative senescence at a very early stage of proliferative cycle rendering it disadvantageous to use the cells for transplantation [3]. Hence, for mainstream therapeutic applications alternate source of MSCs must be considered.

In this regard, human-induced pluripotent stem cells (hiPSCs) reprogrammed from human adult somatic cells, converge to a better-defined ground state of pluripotency. Human-induced pluripotent stem cells can be differentiated into all three germ layer cell types (Ectodermal, Mesodermal and Endodermal) of the organism and – while in the pluripotent state – can be cultured virtually indefinitely without significant signs of replicative senescence. A recent breakthrough in the generation of hiPSCs from human somatic cells by using defined factors, [34, 35] could facilitate generation of patient-specific iMSCs derived from hiPSCs. The iMSCs have the capabilities for utilization in a broad range of regenerative medical applications. Hence, they are often considered as readily accessible promising source of stem cells for future clinical therapies [3]. The iMSCs shared the similar properties compared to the ESC-derived MSCs [33]. Recent studies have also revealed that biomimetic surface results in the rapid and efficient derivation of iMSCs from hiPSCs [36]. However, several challenges need to be effectively tackled before iMSCs could be favourably used for translational applications.

Human pluripotent stem cells (hESCs & hiPSCs) as a novel cell resource for generating clinical-grade products

The PSCs could serve as an alternate source for the generation of MSCs. Embryonic stem cells can be used as an efficient source to generate the MSCs almost indefinitely, attributed to tremendous proliferation potential of ESCs [37]. Nonetheless, ethical concerns, allogenicity and immune reactivity proffer ESC-derived MSCs unsuitable for clinical applications [30]. On the other hand, efficient hiPSCs reprogramming methods could be successfully used to obtain

Table 1 Different types of human somatic cells that have been reprogrammed to induced pluripotent stem cells (hiPSCs)

Cell source	References
Bone marrow MSCs	[38]
Adipose tissue-derived stem cells	[39]
Cord blood cell	[40]
Keratinocytes	[41]
Skin fibroblasts	[41]
Mammary epithelial cells	[42]
Renal epithelial cells	[43]
Corneal epithelial cells	[44]
Peripheral blood cells	[45]
Umbilical cord MSCs	[46]
Placental MSCs	[47]
Amniotic membrane MSCs	[46]
Amniotic fluid-derived cells	[48]

patient-specific iPSCs. Takahashi and Yamanaka were the first groups to demonstrate that mouse [32] and human [34] somatic cells could be successfully converted to iPSCs through the retroviral delivery of Oct4, Sox2, Klf4 and C-Myc. Further characterization of iPSCs indicated human iPSCs are similar to human ESCs in their morphology, gene expression profile, *in vitro* differentiation potential and teratoma formation [30]. Different types of human somatic cells have been successfully shown to reprogram into hiPSCs (Table 1).

The discovery of hiPSCs has accelerated the regenerative medical research [19]. Human-induced PSCs are cells that have the capability of differentiating into all somatic cell derivatives (all three germ layers, for example, ectoderm, mesoderm and endoderm) and also, make a contribution to the germline (Table 2 shows a catalogue of different cell types derived from iPSCs); this unique ability of contribution to chimera and indefinite self-renewal provides a unique opportunity for autologous personalized cell-based therapy [34, 49, 50]. Towards future studies, hiPSCs are considered as the driving force for personalized cell replacement therapy [51].

Establishment of reliable and standardized source of functional MSCs for regenerative applications

The establishment of a reliable source of autologous, transgene-free progenitor cells have enormous potential in the field of cell-based regenerative medicine [3, 4, 30, 33–35], for example, in preparing a therapeutic strategy for infants born with devastating birth defects [73–82]. However, standardization of MSCs remains a major obstacle to the therapeutic usage in regenerative medicine [30, 31, 83]. Comparison of experimental data with different studies becomes difficult when starting materials and culture conditions affects cell preparations [6, 8, 10, 16–18].

Recent studies using RNA-based technology [84], pluripotency-associated protein transfection [85], non-integrating methods of the pluripotent gene containing plasmid usage [86] and a pluripotent gene containing Sendai viral vectors [87, 88] are hinting towards safe clinical usage of footprint-free hiPSC-derived cellular products, such as iMSCs, since these directed differentiated cells will not have any

Table 2 List of different cell types including iMSCs derived from hiPSCs

Cell types	References
Ectoderm	
Neural	[52]
Retinal pigment epithelial cells	[53]
Corneal epithelial cells	[44]
Mesoderm	
Cardiomyocytes	[54]
Adipocytes	[55]
Osteocytes	[56]
Chondrocytes	[57]
iMSCs	[58]
Haematopoietic stem cells (HSCs)	[59]
Erythrocytes	[60]
Platelets	[61]
Endothelial cells	[62]
Neutrophils	[63]
Endoderm	
Lung and airway epithelial cells	[64]
Nephrogenic intermediates	[65]
Follicular epithelial cells	[66]
Hepatocytes	[67]
Kidney progenitor cells	[68, 69]
Pancreatic beta cells	[70]
Germ cells	[71, 72]

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risk of undesired genomic modifications associated with reprogramming protocol.

iMSCs as a novel source of therapeutically active MSCs

The adult MSCs does not exhibit long-lasting immunoregulatory functions *in vitro* and *in vivo* [10]. The primary source of MSCs with high-proliferation potential has been reported as a suitable alternative to the adult MSCs sources [33]. The development of hiPSCs has, in turn, led to the culmination of the unique ability to generate iMSCs by directed differentiation (Table 3). Recent data suggest that iMSCs are emerging as a strong contender for the new sources of MSCs that could be suitable to replace the adult MSCs. Particularly, of late many studies have reported successful derivation of functional MSCs from iPSCs (iMSCs) [33, 36, 50, 58, 89–92]. The iMSCs are a novel class of stem cells that augments effective and reliable regeneration than contemporary methods. The iMSCs can be obtained from the readily accessible adult tissues and exhibit greater proliferation potential than the traditional sources of MSCs [58]. Because of the promising pre-clinical and clinical therapeutic potential of MSCs, the iMSCs derived from iPSCs may serve as an alternate and inexhaustible source [93]. Additionally, the synthetic coating has been shown to assist in the derivation of iMSCs. The derivation of iPSCs into iMSCs on synthetic polymer coating, PMEDSAH [Poly [2-(methacryloyloxy) ethyl dimethyl-(3-sulfopropyl) ammonium hydroxide] resulted in high differentiation efficiency, tri-lineage differentiation potential and expression of characteristic MSCs markers (CD73+, CD90+, CD105+, CD166+, CD31−, CD34− and CD45−) [91]. Similarly, Liu *et al.* has shown that iMSCs could be orderly derived in a single step from iPSCs on fibrillar collagen coating [36]. In a recent study, Chen *et al.* has shown that treatment of iPSCs with SB431542, a transforming growth factor β pathway inhibitor to generate epithelial monolayer-like cells in two-dimensional (2D) culture system followed by induction of epithelial–mesenchymal transition lead to rapid and reliable differentiation into iMSCs [50]. Overexpression of Oct4 along with the combination of GSK3 inhibitor has been demonstrated to reprogram CD34+ peripheral blood or cord blood into functional mesenchymal stromal cells [94].

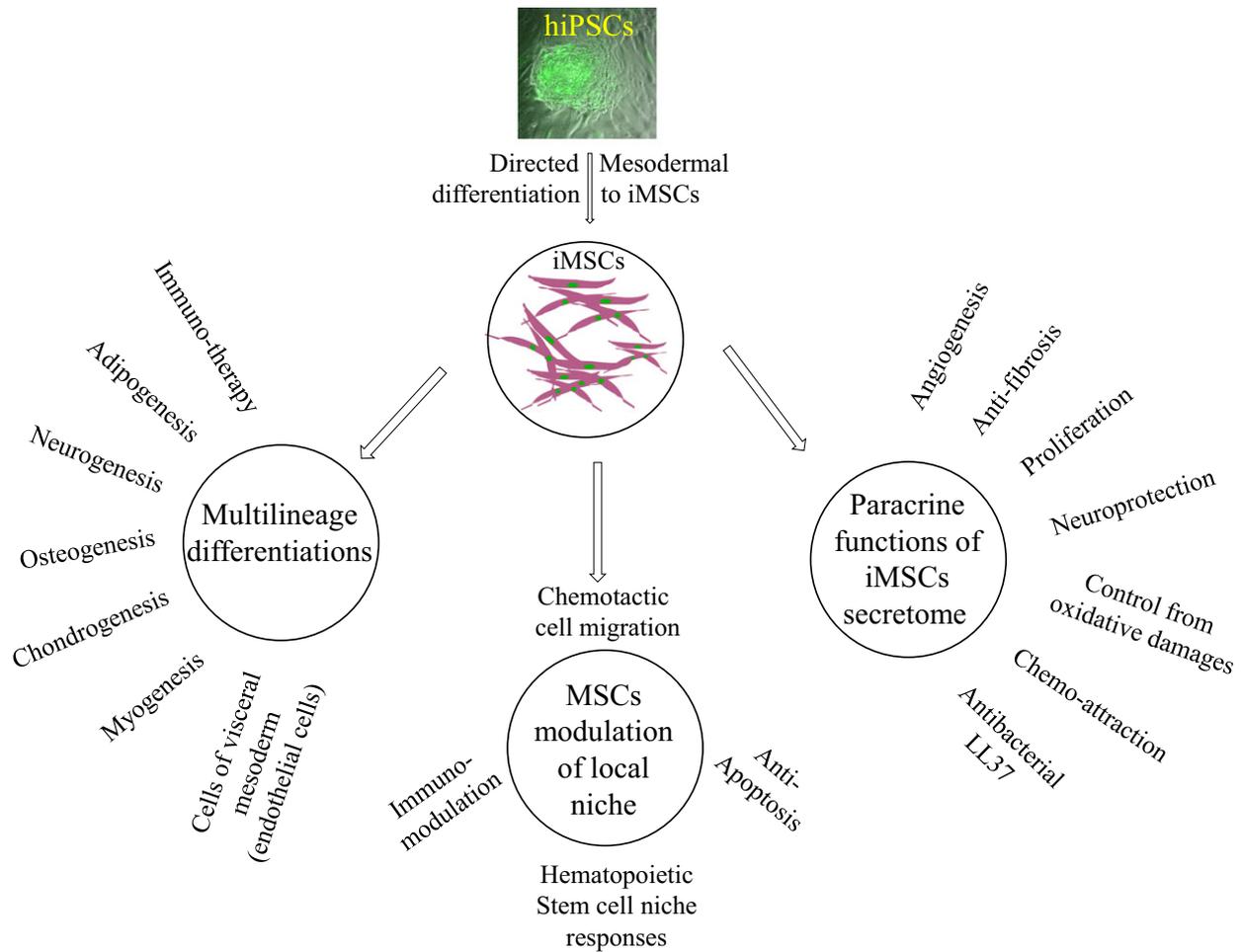## Phenotypic features of iMSCs

The specific cell surface marker on the human MSCs remains to be properly elucidated. Currently, a panel of markers is used to characterize the MSCs isolated from different tissue sources, since there is no specific marker for identifying the bonafide MSCs. The iMSCs satisfies the essential criteria's such as plastic adherence, expression of key MSC surface markers and tri-lineage differentiation capability properties as laid down by the International Society of Cellular Therapy [3]. Himeno *et al.* have demonstrated that iMSCs from mice exhibited characteristic mice MSC surface marker such as CD105, CD140a, Sca-1 and CD44 as previously described [19, 96]. The immunosuppressive, cytoprotection and tissue regeneration properties are exerted by the paracrine factors secreted by the MSCs [97–99]. The iMSCs and ESC-derived MSCs displayed attenuation of proliferation and cytolytic activity of NK cells in a similar way to BMMSCs. The iMSCs offer vast superiority than traditional sources of MSCs, as they can be generated from any tissue source from the body and theoretically iPSCs pose unlimited growth potential. Thus, iMSCs should serve as an inexhaustible source of MSCs [3]. The human MSCs from various tissue sources are typically identified by the expression CD29, CD44, CD73, CD90, CD105, CD146 and CD166. Newer studies have reported that human iMSCs exhibited the above indicated typical characteristics of adult MSCs [33].

Biological characteristics of iMSCs:

The iMSCs and ESC-derived MSCs displayed similar strong immunosuppressive characteristics [33], also iMSCs display a wide range of cytokine profiles, microenvironment modulatory paracrine factors and exert different functions on the local cellular niche components *via* secretion of suitable bioactive molecules (Fig. 1). Giuliani *et al.* further reported that there was no marked functional variability between iMSCs and ESCs–MSCs [33]. Unlike BMMSCs, iMSCs and ESCs–MSCs that could be subjected to long-term culture without resulting in explicative senescence [33]. Studies by Lian *et al.* have shown that iMSCs display typical MSC characteristics and there were no differences between human iMSCs and human ESC-derived MSCs [58, 83]. More robust proliferation was observed in iMSCs than BMMSCs. The iMSCs could be easily scaled up to more than 40 passages while stably maintaining normal diploid karyotype, and consistent gene expression and surface antigen profile [58]. Human iMSCs apart from typical MSCs characteristic markers such as CD29, CD44 and CD73 also expressed a higher level of endogenous pluripotency markers such as Oct4

Table 3 Cell culture supplements that promote *in vitro* derivation of iMSCs from hiPSCs

Materials/Additives	References
Synthetic polymer, PMEDSAH	[91]
Fibrillar collagen	[36]
SB431542, a TGF-β pathway inhibitor	[50]
RGD (Arg-Gly-Asp) peptides	[95]
Fibronectin (Fn)	[95]
Fibronectin-like engineered polymer protein (FEPP)	[95]
Extracellular matrix, Geltrex	[95]
Platelet concentrate	[95]
Oct4	[94]
CHIR99021, GSK inhibitor	[94]

Fig. 1 Intrinsic features of iMSCs, which may allow them to have better biological effectiveness compared to adult hMSCs. Multilineage differentiations may obtain a variety of specialized cells for cell replacement therapy (Table 2 lists different types of cells obtained from hiPSCs). Various cytokines in their secretome profile also help immunomodulation, antifibrotic, anti-apoptotic activities. The microenvironment modulatory paracrine factors may exert a wide range of cellular functions on local cellular niche components *via* the release of the suitable bioactive compound.

[58]. Liu has proposed that iMSCs derived from blood cells could be used as a novel and patient-specific source for usage in disc repair [100]. Comparative study of DNA methylation profiles of iMSCs with normal MSCs and PSCs suggested that iMSCs maintained donor-derived epigenetic differences [101]. In a recent study, published by Frobel *et al.* iMSCs are starter MSCs and subjected to epigenetic analysis. The study indicated that morphology, immunophenotype, *in vitro* differentiation and gene expression of iMSCs were consistent with the initial donor MSCs population. Except iMSCs were impaired in suppressing T-cell proliferation. The iMSCs retained donor-derived DNA methylation (DNAm) profile. However, tissue-specific and age-related DNAm profiles of iMSCs were completely erased. Further, the iMSCs reacquired senescence-associated DNAm. The study also contrastingly highlights that iMSCs reacquire incomplete immunomodulatory functions [102].

Directed Differentiation of iMSCs

The use of stem cells and biologically suitable scaffolds offer the full potential for tissue regeneration. Transplantation of lineage-committed cells can obviate *in vivo* teratoma formation that is caused by the rapid proliferation and uncontrolled spontaneous differentiation of PSCs. Thus, controlled differentiation of hiPSCs into cells that resemble adult MSCs is an attractive approach to obtain a readily available source of progenitor cells for tissue engineering. Unlike previously reported methods that typically rely on the addition of soluble factors to affect PSC differentiation, a recent study by Liu *et al.* reports an alternative approach using a biomaterial coating on a cell culture plate made of fibrillar collagen Type I to promote the derivation of MSC-like cells. This study has reported a collagen matrix that could potentially play a positive role in regulating the differentiation of hESCs and

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hiPSCs towards a multipotent mesenchymal progenitor cell [36]. Activation of epithelial-to-mesenchymal transition (EMT) of epithelial cells has been used successfully by others for generation of MSC-like cells from hESCs [103]. A study by ThienHan et al. to generate MSCs from human iPSCs, and investigate the osteogenic differentiation of iMSCs seeded on biofunctionalized CPCs containing RGD (Arg-Gly-Asp) peptides, fibronectin (Fn), Fibronectin-like engineered polymer protein (FEPP), Geltrex and platelet concentrate has also been reported [80]. A significant part of the study dedicated to the investigation of iMSCs proliferation and osteogenic differentiation atop calcium phosphate cement (CPC) containing biofunctional agents was also evaluated [80].

Therapeutic applications of iMSCs

The iMSCs could be effectively used for diseases modelling, drug screening and therapeutic applications (Fig. 1). The immunological concerns on cell therapy can be effectively bypassed by iMSCs [51]. Nevertheless, the long-term studies on the immunosuppressive activity of the iMSCs remain to be explored [33]. Mesenchymal stem cells are considered as the first line of prophylactic treatment for GVHD and organ transplantation owing to their immunoregulatory properties [27, 104–106]. During allogeneic transplantation, the circulating NK cells target and destroy the graft [107, 108]. On the other hand cotransplantation of MSCs prevent GVHD by attenuating the cytotoxic activity of NK Cells [27, 105, 106]. Under in vitro culture conditions Giuliani et al. has shown that human MSCs derived from the iPSCs considerably down-regulated NK cell cytolytic capabilities [33]. The iMSCs were more potent than the BMMSCs. Thus, iMSCs can be graded as a useful therapeutic option to prevent allograft rejection [33]. The study from Himeno et al. showed MSCs from iPSCs ameliorated diabetic polyneuropathy (DNP) in mice [19]. The results suggest that effects of DNP by MSCs might be because of the secretion of angiogenic/neurotrophic factors and differentiation into Schwann type cells. Mesenchymal stem cells have also been reportedly considered as a potential treatment option for periodontal defects arising from periodontitis. A report by Hynes et al. indicated that iMSCs facilitated the periodontal regeneration coupled with newly formed mineralized tissue in periodontitis rat models [3]. Recently, Yang et al. demonstrated that tumour necrosis factor alpha-stimulated gene-6 (TSG6) expressing iMSCs were capable of decreasing the inflammation in experimental periodontitis model and inhibiting alveolar bone resorption [109]. Human MSCs have emerged as a promising therapeutic source for treating myocardial and limb ischaemia [110, 111]. An investigation by Lian et al. revealed that human iMSCs attenuate limb ischaemia in mice [58]. Further analysis showed that transplantation of iMSCs into mice exhibited better attenuation in hindlimb ischaemia than adult BM-MSCs. The greater therapeutic efficacy can be attributed to their ability to survive for a longer time after transplantation. Tracking of transplanted iMSCs divulged, iMSCs could engraft and survive for more than 5 weeks following transplantation [58]. Wei et al. indicated human iMSCs could continuously proliferate for more than 32 passages without undergoing cellular senescence and displayed superior wound healing and pro-angiogenic properties

[92]. The iMSCs derived on a synthetic polymeric coating; PMEDSAH resulted in novel bone formation when transplanted into the mice with calvarial defects [91]. Zang et al. have shown that iMSCs derived from Hutchinson–Gilford Progeria syndrome (HGPS) were helpful in studying the molecular pathology of HGPS [89]. In a recent study, Liu et al. has successfully utilized iMSCs for modelling Fanconi anaemia. The Fanconi anaemia iPSC-derived MSCs displayed premature senescence [112]. Also, hiPSC-derived cells have been successfully used to model various other diseases. We have provided the comprehensive details below in Table 4, a list of disease modelling using hiPSCs (Fig. 2).

Recent developments in safe clinical products

Challenges & strategy to overcome them for Clinically Relevant iMSCs

Safety and Efficacy of iMSCs are of paramount importance to succeed in the field of translational regenerative medicine. The viral vector-based strategy for reprogramming might result in tumour formation as a result of insertional mutagenesis of the transgene. C-Myc is a proto-oncogene that has been shown to increase the efficiency of reprogramming by suppressing the tumour suppressor p-53 gene. The overexpression of proto-oncogene and moderating of tumour suppressor genes render hiPSCs beneficial results regarding higher proliferative advantage for downstream translational applications [206]. Consequently, many studies have unveiled several different strategies for generation of safer iPSCs. In 2010, Yamanaka's group suggested using L-Myc as an alternative to C-Myc for reprogramming based on the result that L-Myc maintained in the reprogramming efficiency without inducing any tumorigenesis [206]. Fang et al., reported generated iPSCs devoid of C-Myc enervated retinal ischaemia and reperfusion injury following transplantation in rat models [207]. The starter cell type has an enormous impact on reprogramming, differentiation and in vivo functionality because of epigenetic memory. Until now, there are no data on the best starter cell type for a particular clinical application. Hence, more research needs to be conducted to determine suitable starter cell type based on the type of clinical application [30]. While iPSC-derived cell source are emerging as a replacement cell source, their traits of self-renewal and pluripotency after in vivo transplantation often leads to tumorigenicity and genomic instability might result in low clinical utility [3]. The nature of pluripotency transgene elements present in the iMSCs is arduous to predict. Hence, iMSCs have been thoroughly characterized for silencing of the transgene expression and safe transgene integration [33]. Presently, only initial studies are reported on preclinical applications of iMSCs. Hence, long-term, multicentric, pre-clinical and clinical studies are required for accurate prediction of iMSCs for the translational purpose [33]. The recent development of non-viral-based generation of iPSCs might pave the way for considering iPSCs as a suitable candidate for biotherapeutics [86, 208–210]. Newer

Table 4 Disease modelling using hiPSCs

Disease modelling		References
Neurological		
Development	Fragile X/ataxia syndrome (FXA)	[74, 113]
	Rett syndrome (RS)	[75]
	Angleman syndrome	[76]
	Prader–Willi syndrome	[76]
	Timothy syndrome (TS)	[77]
	Microcephaly (MC)	[78]
	Hereditary spastic paraplegias (HSP)	[79]
	Olivopontocerebellar atrophy (OPCA)	[80]
	Pelizaeus–Merzbacher disorder (PMD)	[81]
	Mitochondrial encephalopathy with lactic acidosis and stroke-like episodes (MELAS)	[82]
	Glioblastoma iPSCs	[114]
	Childhood cerebral adrenoleukodystrophy (CCALD)	[115]
	Multiple sclerosis	[116]
	Autism spectrum disorder (ASD)	[117]
	Cernunnos deficiency syndrome (XLF)	[118]
	William–Beuren syndrome (WBS)	[119]
	William–Beuren region duplication syndrome (WBDS)	[119]
Degenerative	Alzheimer's (AD)	[120–122]
	Schizophrenia (SCZD)	[123]
	Spinal muscular atrophy (SMA)	[124]
	Parkinson disease (PD)	[125–127]
	Huntington disease (HD)	[125, 128]
	Amyotrophic lateral sclerosis (ALS)	[129]
	Familial dysautonomia (FD)	[130]
	X-linked adrenoleukodystrophy (X-ALD)	[131]
	Machado–Joseph disease (MJD)	[132]
	Friedreich's Ataxia (FRDA)	[133]
	Familial transthyretin amyloidosis (ATTR)	[134]
	Tauopathies (TAP)	[135]
	Diabetic polyneuropathy (DPN)	[19]

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Table 4. Continued

Disease modelling		References
	Gaint axonal neuropathy (GAN)	[136]
	Menkes disease (MD)	[137]
	Frontotemporal dementia (FTD)	[138, 139]
	Spinal cerebral ataxia type2 (SCA2)	[140]
	Ataxia telangiectasia (AT)	[141]
	Dravet syndrome (DVS)	[142]
Hematological	Swachman–Bodian–Diamond syndrome (SBD)	[125]
	Adenosine deaminase deficiency (ADA) severe combined immunodeficiency (SCID)	[125]
	Fanconi anemia (FA)	[143]
	Sickle cell anaemia (SCA)	[144]
	Beta-thalassaemia (BT)	[145]
	Polycythaemia vera (PV)	[146]
	Congenital amegakaryocytic thrombocytopenia (CAMT)	[147]
	Paroxysmal nocturnal haemoglobinuria (PNH)	[148]
	Dyskeratosis congenita (DC)	[149]
	α-Thalassaemia (AT)	[150]
	Aplastic anaemia (AA)	[151]
	Myeloproliferative disorder (MPN)	[152]
	Chronic myeloid leukaemia (CML)	[153]
	Juveline myelomonocytic leukaemia (JMML)	[154]
	Chronic infantile neurological, cutaneous and articular syndrome (CINCA)	[155]
	X-linked chronic granulomatous disease (X-CGD)	[156]
	Severe congenital neutropaenia (SCN)	[157]
	Wiskott–Aldrich syndrome (WAS)	[158]
Metabolic	Gaucher disease type III (GD)	[125]
	Juvenile diabetes mellitus (JDM)	[125]
	Lesch–Nyhan syndrome (LNS)	[125]
	Aplha1-Antitrypsin deficiency (A1ATD)	[159]
	Pompe disease (PomD)	[160]
	Familial hypercholesterolaemia (FH)	[161]
	Tyrosinaemia (TYS)	[162]
	Glycogen storage disease type1 (GSD)	[162]

Table 4. Continued

| Disease modelling		References
	Progressive familial cholestasis (PFD)	[162]
	Crigler–Najjar syndrome (CN)	[162]
	Hurler syndrome (HS)	[163]
	Neuronal ceroid lipofuscinosis (NCL)	[164]
	Wilson's disease (WD)	[165]
	Mitochondrial diabetes (MT)	[166]
	Fabry disease (FD)	[87]
	Mucopolysaccharidosis type IIIB disease (MPS)	[167]
Cardiovascular	LEOPARD syndrome (LS)	[78]
	Long QT syndrome type 1 (LQTS1)	[168]
	Long QT syndrome type 2 (LQTS2)	[169]
	Long QT syndrome type 3 (LQTS3)	[170]
	Supervascular aortic stenosis (SVAS)	[171]
	Hypertrophic cardiomyopathy (HCM)	[172]
	Diabetic cardiomyopathy (DCM)	[173]
	Hypoplastic left heart syndrome (HLHS)	[174]
	Moyamoya disease (MMD)	[175]
	Catecholaminergic polymorphic ventricular tachycardia (CPVT)	[176]
	Familial dilated cardiomyopathy (DCM)	[177]
	Familial hypertrophic cardiomyopathy (HCM)	[178]
Primary immunodeficiency	SCID/Leaky SCID	[179]
	Omenn syndrome (OS)	[179]
	Cartilage–hair hypoplasia (CHH)	[179]
	Herpes simplex encephalitis (HSE)	[179]
Musculoskeletal disorder	Craniometaphyseal dysplasia (CMD)	[88]
	Duchenne muscular dystrophy (DMD)	[125]
	Becker muscular dystrophy (BMD)	[125]
	Osteogenesis imperfect (OI)	[180]
	Thanatophoric dysplasia (THD)	[181]
	Achondroplasia (ACH)	[181]
	Hutchinson–Gilford progeria syndrome (HGPS)	[182]
	Werner syndrome (WS)	[183]

Table 4. Continued

Disease modelling		References
	Facioscapulohumeral muscular dystrophy (FSHD)	[184]
	Limb-girdle muscular dystrophy (LGMD)	[185]
	Myotonic dystrophy type 1 (MyD1)	[186]
	Marfan syndrome (MFS)	[187]
	Fibrodysplsia ossificans progressiva (FOP)	[188]
Lung disorder	Cystic fibrosis (CF)	[189]
	Pulmonary alveolar proteinosis (PAP)	[190]
	Emphysema (EP)	[191]
Dermatological Disorder	Recessive dystrophic epidermolysis bullosa (RDEB)	[192, 193]
	Scleroderma (SC)	[191]
	Focal dermal hypoplasia (FDH)	[194]
	Hermansky–Pudlak syndrome (HPS)	[195]
	Chediak–Higashi syndrome (CHS)	[195]
Cancer	Breast cancer (BC)	[196]
Opthalmological disorder	Retinitis pigmentosa (RP)	[53, 197, 198]
	Gyrate atrophy (GA)	[199]
	Best disease (BD)	[200]
	Cataract (Cat)	[201]
	Ectrodactyly-ectodermal dysplasia-cleft syndrome (EEC)	[202]
Nephrology	End stage renal disease (ESRD)	[203]
Aneuploidy	Turner syndrome (TS)	[204]
	Warkany syndrome (WKS)	[204]
	Patau syndrome (PS)	[204]
	Emanuel syndrome (ES)	[204]
	Klinefelter's syndrome (KS)	[205]
	Down's syndrome	[125]

technologies without viral transgene such as chemicals, plasmids and recombinant protein-based approaches might augment the clinical utilization of these safe iPSCs [85, 211, 212]. The low efficiency of iPSCs generation might be a serve-debilitating factor to consider iPSCs/iMSCs for translational applications. Hence, more research needs to be focused on scaling and optimizing the quality of iPSCs [58].

Regulatory issues for future safe therapies using hiPSCs

The iPSCs present unique sets of technical and regulatory hurdles when compared to even ESCs for translational applications [213]. The issues regarding the cell and gene therapy in every country are governed by its sovereign regulatory body. In the United

Fig. 2 Potential application overview of iMSCs derived from hiPSCs.
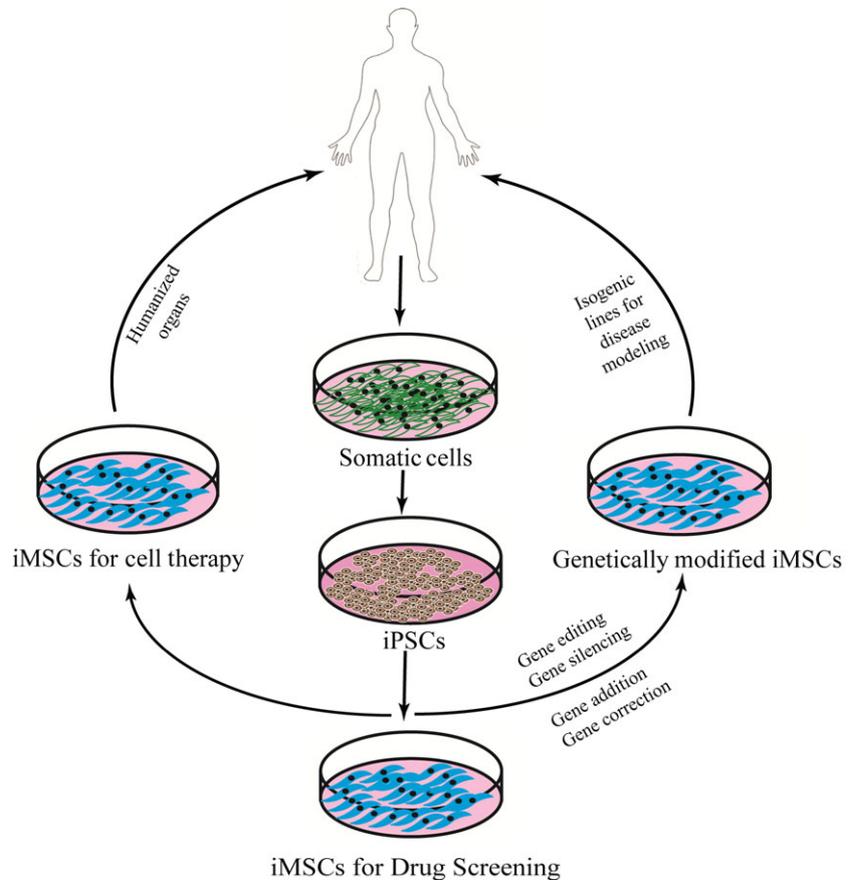
States, the human iPSC products are regulated by Centre for Biologics Evaluation and Research at the United States Food and Drug Administration (USFDA) [214]. Before proceeding with the clinical trials, the iPSC-derived products are subjected to preclinical testing that requires extensive examination of safety, feasibility and efficacy [215]. Pre-clinical studies involve a comparative analysis of the various parameters between healthy animals and disease models. According to the FDA guidelines, the same cells used in preclinical trials should be used during clinical trials [215]. Small animal models, such as rodents are used in preclinical studies. However, rodent models, although could be used for basic biological studies have a poor predictive outcome in term of clinical efficacy [215]. Consequently, pre-clinical studies consisting of large animal models such as swine, primates, *etc.*, are favourable as they have relatively longer life span and displays physiological similarities to humans albeit a limited number of disease models and inability to modify the genome with ease constitute major road block in the usage of large animal models [215]. For a particular disease condition, a single satisfactory model is not present. Hence, pre-clinical testing must be carried out using suitable alternative models to highlight potential limitations and assist in finding suitable alternative avenues for handling the disorder [215]. Necessary precautions must be undertaken before extrapolating the results from animal models to clinical trials [215].

Efficacy of the transplanted cells *in vivo* is not well documented. A few studies have demonstrated that transplantation of PSCs and differentiated cells resulted in poor survival of the cells [216–219]. The fate of the transplanted cells must be evaluated to ascertain the tangible effectiveness of the cells *in vivo* following transplantation. Hence, suitable surgical/imaging techniques should be developed for *in vivo* fate mapping of the cells [215].

Current good manufacturing protocol guidelines must be followed to generate and characterize iPSC-based products [215] for any future clinical applications. The quality of cell products and homogeneity of the cell population will determine the effect, risk and potency of the iPSC-based therapy [215]. Method and duration of storage, viability, cell line contamination and risks of transmissible infections are some of the other possible confounding factors that can affect the cell therapy [215]. Before scaling up towards clinical trials questions such as ideal cell source, efficient reprogramming and differentiation protocols, demonstration of safety and functionality have to be addressed [215].

Generation of iPSCs from somatic cells requires a significant amount of molecular manipulations [213] either by viral vectors containing reprogramming genes [34] or transfection of

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reprogramming mRNAs [84] or purified reprogramming factors [85] or transfection by non-viral vectors containing reprogramming gene methods [86]. The viral-based reprogramming strategies form the basis of added concerns because of random integration into the host genome [34]. On the contrary use of retroviral-based genetically modified cells is technically permitted for human clinical trials under the existing National Institutes of Health (NIH), guidelines [213]. Recent strategies for using small molecule-based reprogramming & differentiation must be explored to develop and differentiate into clinical relevant cell types [220]. Generation of iPSCs require a significant amount of manipulations [213]. The viral-based reprogramming strategies form the basis of added concerns because of random integration into the host genome [34]. On the contrary, use of retroviral-based genetically modified cells is technically permitted for human clinical trials under the existing NIH, guidelines [213]. Recent strategies for using small molecule-based reprogramming & differentiation must be explored to develop and differentiate into clinical relevant cell types [220]. Every iPSC line would exhibit unique genetic and epigenetic constitution. Hence, each and every cell line has to be subjected to independent characterization to determine its precise characteristic features [213]. It is necessary to determine to what extent iPSCs are similar to ESCs. Besides safety, efficacy, stability, heritability and absence of biased lineage differentiation have to examine and documented [213].

Immune response against transplanted cells presents a critical challenge that can detrimentally affect the outcome of therapy. Some of the important questions as to why the donor cells pose a risk of immune response or genetic diseases, cell efficiency, cells exhibiting risk of contamination, effectiveness and safety of transplanted cells has to be answered, if they are perceived to develop into potential therapeutic agents [221–223]. Tumour formation remains one of the most important concerns while using the pluripotent cells or PSC-derived products. It has been well documented that the reminiscent PSCs present in differentiated cells could effectively give rise to tumour formation [217, 219, 224–228].

One of the biggest advantages of iPSCs is the possibility of generation of patient-specific autologous cell lines. Hence, the cumbersome procedure of screening against different cell lines for a proper match is excluded [34, 213, 229]. The method of selection and characterization criteria needs good manufacturing protocol [213]. The combination of a proven gene therapy with proven PSC-derived products might hold a great potential for therapeutic application albeit certain technical and regulatory hurdles. Hence, suitable regulatory guidelines should be established for the application of genetically modified stem cells [213].

Conclusion

The invention of cellular reprogramming of adult cells from the terminally differentiated state of PSCs state with the help of transcription factors, biological factors and small molecules open up a large window of opportunity in the field of regenerative medicine. By incorporating the advantages of both iPSCs and MSCs, the resulting iMSCs are emerging as a novel stem cell population [3]. The iMSCs generated from iPSCs successfully exhibited all the fundamental criteria for defining the MSC population based on the existing knowledge [3]. Data indicate that iMSCs can be used as a promising alternative strategy for treatment of various immune-mediated diseases [33]. Although, the concept of iMSCs is at its nascent stage, recent studies nevertheless provide the proof of concept that functional iMSCs could be successfully generated from iPSCs that exhibit robust proliferation and differentiation potential, which could be used for tissue repair and engineering applications [58]. The development of iMSCs offers promise of patient-specific, cost-efficient and batch to batch consistency [58]. Presently, the scope of iMSCs is limited to the pre-clinical utility for tissue engineering-based treatment approaches. Further pre-clinical and clinical studies are required before scaling it towards routine clinical utility.

Acknowledgements

Conflicts of interest

None.

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