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Review

Placenta: A gold mine for translational research and regenerative medicine

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ABSTRACT

Stem cell therapy has gained much impetus in regenerative medicine due to some of the encouraging results obtained in the laboratory as well as in translational/clinical studies. Although stem cells are of various types and their therapeutic potential has been documented in several studies, mesenchymal stromal/stem cells (MSCs) have an edge, as in addition to being multipotent, these cells are easy to obtain and expand, pose fewer ethical issues, and possess immense regenerative potential when used in a scientifically correct manner. Currently, MSCs are being sourced from various tissues such as bone marrow, cord, cord blood, adipose tissue, dental tissue, etc., and, quite often, the choice depends on the availability of the source. One such rich source of tissue suitable for obtaining good quality MSCs in large numbers is the placenta obtained in a full-term delivery leading to a healthy child's birth. Several studies have demonstrated the regenerative potential of human placenta-derived MSCs (hPMSC), and most show that these MSCs possess comparable, in some instances, even better, therapeutic potential as that shown by human bone marrow-derived (hBMSC) or human umbilical cord-derived (hUC-MSC) MSCs. The placenta can be easily sourced from the OB/GYN department of any hospital, and if its derivatives such as hPMSC or their EVs are produced under GMP conditions, it could serve as a gold mine for translational/clinical research. Here, we have reviewed recent studies revealing the therapeutic potential of hPMSC and their extracellular vesicles (EVs) published over the past three years.

Contents

1. Introduction

Stem cells can be broadly divided into two main categories: embryonic and adult. Embryonic stem cells (ESCs) are derived from the inner mass of a blastocyst [1,2] Adult stem cells comprise all tissue-specific stem cells like hematopoietic stem cells (HSCs),

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mesenchymal stem cells (MSCs), neural stem cells (NSCs), muscle stem cells (MuSCs), hair root stem cells (HRSCs), etc. Adult stem cells have a shorter replicative span, and thus, provide only a limited supply of cells. Fetal tissues, by contrast, provide cells that have rapid cell growth, better multi-differentiation capacity, and are less likely to be rejected [3]; however, ethical concerns associated with this source [4] make this precious source of cells unavailable, or at least hard to procure, to the researchers. Nonetheless, fundamental and translational research is being done on adult stem cells and very encouraging data are emerging from the studies.

Although stem cells are of various types and their therapeutic potential has been documented in several studies, mesenchymal stromal/stem cells (MSCs) have an edge. In addition to being multipotent these cells pose fewer ethical issues, have an immunomodulatory property and possess regenerative potential compared to other stem cells isolated from various sources. Currently, MSCs are being sourced from various tissues such as bone marrow, cord, cord blood, adipose tissue, dental tissue, etc., and, quite often, the choice depends on the availability of the source, and it is not based on comparative studies between MSCs obtained from different sources but on tissue availability. The placenta obtained from full term delivery is a good source of tissue suitable for obtaining good quality MSCs in large numbers. Several studies have demonstrated the regenerative potential of human placenta mesenchymal stromal cells (hPMSC), and most show that these MSCs possess comparable [5–10], in some instances even better [11–13], therapeutic potential as that shown by human bone marrow mesenchymal stem cells (hBMSC) or human umbilical cord mesenchymal stem cells (hUC-MSC). The placenta can be obtained from the OB/GYN department of any hospital, and if its derivatives such as hPMSC and their EVs are produced under GMP conditions, it could serve as a gold mine for translational/clinical research (Graphical Abstract and Fig. 1).

Since we specifically wanted to highlight the latest developments in the field, we have limited this review to the recent research (2018–2020) on hPMSC and their extracellular vesicles (EVs).

1.1. Placenta

A placenta obtained in a full-term delivery leading to the birth of a healthy baby is an attractive source of hPMSC for regenerative medicine as it is a readily available tissue, can be obtained non-invasively and poses fewer ethical issues when compared with other sources like bone marrow, cord blood, etc. [14]. In addition to MSCs, the placenta can also be used to obtain various products such as extra-cellular vesicles (EVs) – isolated from *ex vivo* cultured hPMSC (hPMSC-EVs) [15,16], growth factors, placental extracts, etc. [17]. The placental extract is already in the market for wound healing applications (e.g., Placentrex, Albert David). The amniotic fluid can also be used to obtain MSCs (hAF-MSC) and EVs (hAF-MSC-EVs). Likewise, amniotic membranes have been used to repair cartilage damage [18], nasal reconstruction [19], and psoriasis [20].

The structure of the placenta and applications of placental derivatives in clinical situations have been extensively reviewed [21,22,14,17], and hence, these aspects have not been reiterated here. The present review is focussed on the recent pre-clinical and clinical studies done with hPMSC and hPMSC-EVs in pre-clinical or translational/clinical setup.

2. hPMSC

An international workshop was held in Brescia, Italy, to define, as clearly as possible, the region of origin and methods of isolation of cells derived from the placenta, especially considering the complexity of the placenta and phenotypic and functional differences observed in the MSCs generated from its various parts. Some

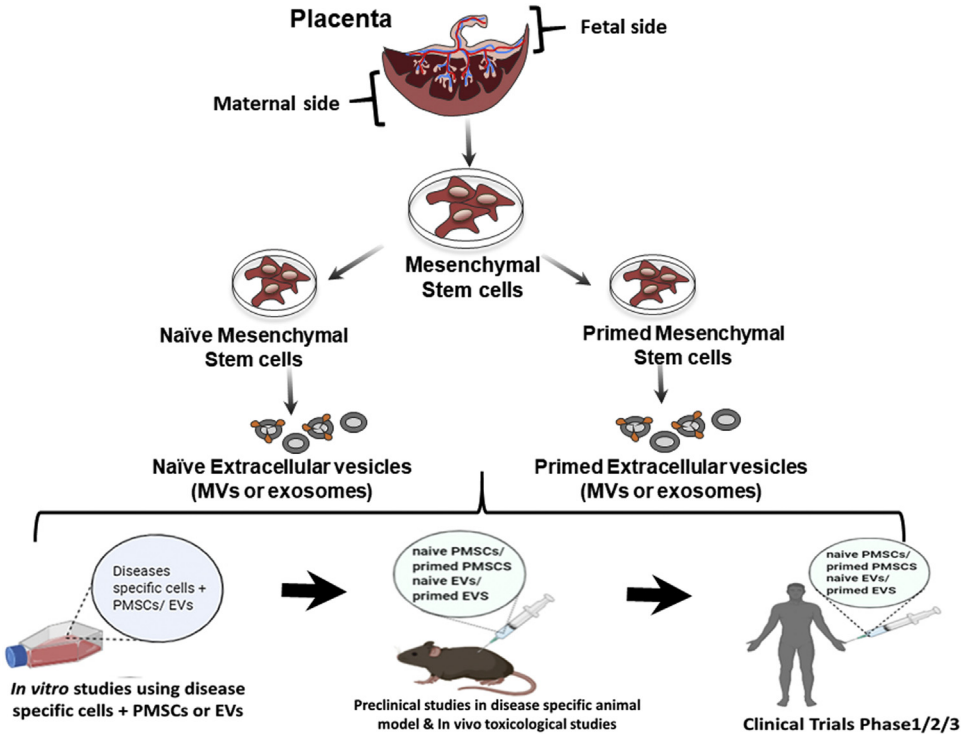

Fig. 1. The figure illustrates various types of studies being done with mesenchymal stromal cells isolated from human placenta (hPMSC) or the extracellular vesicles (EVs) isolated from them. The hPMSC have been used as such (naïve) or have been primed with pharmacological or genetic means (primed) to boost their regenerative properties for specific applications. Likewise, EVs isolated either from naïve hPMSC or the primed ones are being used.

Table 1
In vitro studies done using various placenta and related tissue derived mesenchymal stem cells types.

Sr No	Intervention /Condition/experimental system	Outcome & critical observations	Refs.
1.	Anti-oxidative stress response in endothelial cells	H_2O_2 (100 μM) treated HUVEC when cultured with hBD-MSC showed a reduced expression of genes associated with inflammation and oxidative stress. These data indicate that hPMSC could be potentially used to alleviate the stress in endothelial cells. These results need to be investigated in a suitable animal model.	42
2.	High glucose-induced damage to human endothelial cells	Mesenchymal stem cells derived from human decidua basalis (hBD-MSC) or their conditioned media when added to high glucose treated HUVECs led to some reduction in the expression of genes associated with elevated glucose. The authors did not consider a possibility of dilution of glucose by addition of conditioned medium, thereby reducing its detrimental effect. Surprisingly, HUVECs proliferated even at 0 mM glucose containing media for 72 h. Some genes associated with endothelial cell injury were upregulated in hBD-MSC conditioned media, which is opposite of the claim made by the authors. It is not clear if high glucose levels in media induced any damage in endothelial cells at functional level.	43
3.	Reduction of inflammation in human middle ear epithelial cells due to particulate matter	Human middle ear epithelial cells (hMEEC) exposed to 300 μg/ml of particulate matter (PM) were co-cultured with hPMSC. After co-culture for 4 days, hMEECs showed a reduced expression of pro-inflammatory cytokines - TNF-α, IL-6, MUC-5B and IL-1β. However, apart from reduction in gene expression of pro-inflammatory cytokines by qRT-PCR, whether PM indeed caused any damage to hMEECs was not demonstrated by any functional assay, hence, it could not be established whether hPMSC truly reduced the damage caused by PM in hMEECs.	44
4.	*In vitro* human intestinal fibrosis model	Non-cancerous human intestinal cells were stimulated with TGF-β, which led to overexpression of pro-fibrosis genes such as Collagen1, FN and αSMA. When these cells were co-cultured with hPMSC (2×10^5 cells) and hUC-AMSC (2×10^5 cells) the profibrosis gene reduced. CCG-100602 (MRTF-A/SRF inhibitor) when used on intestinal cells reduced the expression of pro-fibrosis genes in response to TGF β1. Co-culture of hPMSC and hUC-AMSC also showed reduction in MRTF-A, SRF, RHO and ROCK, thus suggesting that hPMSC and hUC-AMSC prevented the emergence of intestinal fibrosis by regulating the expression of MRTF-A.	45
5.	Regulation of T cells by hPMSC and hAMSC	The authors found, as expected, that fetal-derived stem cells were more effective in T cell modulation. To understand effect of MSCs on T cell proliferation, hPMSC and hAMSC were irradiated and then co-cultured with (1×10^5 cells) allogeneic PBMC in three ratios PBMC: MSC ratios (1:1, 1:0.5 and 1:0.1). At all the ratios hPMSC led to proliferation of CD4 and CD8 cells, while hAMSC allowed T cell proliferation only at low ratio (1:0.1). The cytotoxic cell number was more when T cells were co-cultured with hPMSC. Both hPMSC and hAMSC prevented monocyte differentiation. The results must be viewed keeping in mind that MSCs exert their effects via their secretome, and irradiation could have altered their secretome. Whether the physiological secretome would also elicit the same T cell regulatory effect needs to be examined.	24
6.	Immunomodulation of T cells via FoxP3 gene	Comparison between effect of hPMSC and hBMSC on T regulatory cell maturation and function from peripheral blood naïve T cells was performed. T cell activation was similar in both hPMSC and hBMSC sets. FoxP3 gene activation is crucial for T regulatory formation; both hPMSC and BMSC were able to activate FOXP3 in naïve T cells. Co-culture of peripheral blood T cells with hPMSC showed slightly more number of $CD4^+ CD25^+ FoxP3^+$ cells than co-culture with BMSC. Thus, hPMSC are comparatively more efficient at modulating the T cells.	46
7.	Placenta-derived (hPMSC) and amniotic fluid-derived MSC (hAF-MSC) regulate Treg cell formation and cytokine secretion	MSC derived from human amniotic fluid (hAF-MSC), bone marrow (hBMSC) and placenta (hPMSC) were compared for their immunomodulatory properties. Stimulated PBMCs were seeded onto mitomycin C inactivated hAF-MSC, hPMSC and hBMSC (2×10^4 cells of each). Amongst the three hMSCs used, hPMSC reduced the levels of TNFα, IL-1β, IFN-γ, and NF-κB. Even though hAF-MSC showed similar reduction in lymphocyte migration, only hPMSC were used to investigate this mechanism of suppression. Compared to PHA treated hPMSC, the hPMSC reduced the percentage of Th1 and Th2 cells, and increased the percentage of Treg cells.	10
8.	*Ex vivo* corneal cultures to examine MSC efficacy to reduce corneal scar	Post-mortem human corneas were used to set up organ cultures for 28 days. Once the corneas were assigned into control and transplant group, corneal injury (superficial keratectomy) was created. To the transplant group corneas 3×10^5 hPMSC were injected intrastromally. Haematoxylin and Eosin staining of the corneal sections revealed that only hPMSC-treated corneas had organized bowman's capsule. The transplanted corneas showed slightly less laser light scattering, as compared to the control corneas, suggesting hPMSC-mediated reduction in scarring. An animal model for corneal injury model should be used to confirm this finding.	47
9.	hPMSC effect on mitochondrial function in trophoblast cells	Human trophoblast obtained from early pregnancy placenta were seeded (1×10^5 cells/well) in the upper chamber of the Trans well system and hPMSC ($2.5\text{-}\times 10^5$ cells/well) were seeded in the lower chamber. Co-culture was set up for 48 h. hPMSC enhanced the invasion ability of trophoblast cells via upregulation of HIF1α, MMP2/9 and Rho signalling pathway. hPMSC increased PARKIN expression that led to mitochondrial autophagy. However, it is not clear how increased mitochondrial autophagy resulted in an increased ATP production and this needs to be investigated.	48
10.	Effect of hPMSC on IL-6 level in PBMCs from asthmatic children	The asthmatic children were grouped based on the IgE levels and their PBMCs were collected. PBMC (2×10^5 cells/mL) from non-asthma group were cultured with $1/10^{th}$ hPMSC, while PBMC from asthma group were co-cultured with hPMSC in $1/5^{th}$ to $1/20^{th}$ level. The cultures were incubated for 72 h. Regardless of the lower or higher cell number used, hPMSC reduced IL-5 expression to the same extent. Even with the different ratios of hPMSC:PBMC the reduction in IL-5 was similar. Thus, hPMSC could be used to reduce inflammation in asthmatic patients.	49

of the criteria to define MSCs derived from the placenta, namely, amniotic MSC (hAMSC) and chorionic MSC (hCMSC) were laid down. Except that these MSCs should be of fetal origin, which is an important parameter, especially in the context of immuno-regulatory aspects, other criteria were the same as those defined for MSCs from any other tissues [21]. A comparative study done with hPMSC derived from fetal and maternal regions of the placenta showed that, though both showed immuno-modulatory activity, fetal cells exhibited a stronger potential [24].

Cultured hPMSC typically show fibroblastic morphology, adhere to plastic, contain fibroblast colony-forming units, possess a specific pattern of surface antigen expression (positive for CD90, CD73, CD105; negative for CD45, CD34, CD14, and HLA-DR); and have differentiation potential towards osteogenic, adipogenic, chondrogenic, and vascular/endothelial lineages [21,23].

Several insightful reviews have appeared on the therapeutic applications of hPMSC for specific conditions like sports injuries, acute liver failure, Bronchopulmonary Dysplasia, therapeutic angiogenesis, etc. [25–28]. Likewise, Teofili et al. (2018) reviewed the clinical studies – most of which mainly show safety, rather than efficacy, of the cells – as well as pre-clinical studies done using hPMSC to underscore the need of including placental tissue cells in cord blood banking [29]. From the literature reviewed in these articles, it appears that the high diversity of hPMSC resulting from

their isolation from various regions of the placenta – a complex tissue – perhaps restricts the studies of hPMSC to safety-related clinical trials and delays the efficacy-determining ones [30,31]. Since hPMSC derived from different regions of the placenta have been shown to exhibit different properties, at least with respect to their tri-lineage differentiation, [32], not only the region of the placenta used to derive MSCs needs to be documented in every study but safety, as well as efficacy trials of these region-specific MSCs to be used for particular applications need to be carried out so that the field moves further. Some of the studies have taken cognizance of this aspect [30,31]. In addition, Silini et al. (2019) have specifically reviewed the clinical applications of human amniotic membrane-derived MSCs (hAMSC), including their immunomodulatory and antimicrobial properties, and have discussed their role in tumor progression [33]. Here we have mentioned the region of placenta used to derive the hPMSC used in various studies, wherever such explicit mention was found. We have used the nomenclature as per the consensus for naming the placenta and perinatal derivatives [34].

It is worth noting here that the mothers' conditions like diabetes, preeclampsia, gestational diabetes mellitus (GDM), obesity, etc., and their age are reflected in the hPMSC's functionality and affect their therapeutic potential [35–40]. GDM and preeclampsia are complications that commonly occur in pregnancy. Chen et al. (2019) found that despite showing normal phenotype and morphology, the human chorionic membrane MSCs (hCP-MSC) grown from the placentae collected from mothers suffering from GDM (GDM-hCP-MSC) showed increased adipogenic activity, indirectly indicating the loss of osteogenic properties and a reduced effect on macrophage regulation. Thus, this study suggested that gestational diabetes reduces differentiation potential and compromises immuno-regulatory functions of MSCs [37]. Hence, GDM-hPMSC would not be suitable for applications requiring immuno-modulation. Qu et al. (2018) found that human basal decidua MSCs (hBD-MSC) isolated from the placenta of mothers suffering from preeclampsia (PE- hBD- MSC) showed an upregulation of miR-222 and down-regulation of BCL2L11, and consequently, underwent apoptosis when exposed to hypoxia [36]. In other words, these MSCs would not be suitable for the treatment of ischemia-induced pathologies. On the other hand, Basmaeil et al. (2020) found that PE-BD-MSC lacked the expression of HMX-1, a molecule needed to combat oxidative stress. They further showed that PE-BD-MSC exhibited a decreased proliferation, migration, adhesion, and clone formation potential compared to their normal counterparts [39]. Expression of HMX-1 and PE-BD-MSC's functionality could be rescued by pre-conditioning them with low doses of H_2O_2. Loss of HMX-1 would make these cells unable to face oxidative stress in recipients' bodies and would perhaps fail to give requisite therapeutic effect unless primed with compounds like H_2O_2. However, these findings need to be confirmed by *in vivo* studies.

These studies underscore the importance of ensuring that the placentae are collected from healthy mothers giving birth to full-term healthy babies. To ascertain this, mothers' clinical parameters need to be precisely noted, and these data should be considered an important QA/QC parameter for banking of clinical-grade hPMSC and their derivatives. Nonetheless, the hPMSC obtained from placentae collected from deliveries of mothers having such conditions could be harnessed as disease models for fundamental research. Such disease models could yield important information about the disease's pathophysiology, and thus, could pave the way to the development of therapeutic approaches.

We have stratified the studies done with hPMSC into various categories, such as *in vitro* studies using cells collected from healthy individuals or patients suffering from specific disorders/disease, *in vivo* studies done in suitable animal models, and clinical trials (Fig. 1). In addition to reviewing these studies, we have also reviewed the studies wherein hPMSC had been primed with specific agents to improve their therapeutic properties [41].

2.1. In vitro *studies using cells of human origin*

Human umbilical vein endothelial cells (HUVECs) treated with various stressors is a popular model of endothelial dysfunction seen in many diseases like diabetes, atherosclerosis, etc. Atherosclerosis is characterized by endothelial activation due to the accumulation of high amounts of low-density lipoprotein (LDL) and immune cells that lead to the production of oxidative stress mediators, such as H_2O_2. Alshabibi et al. (2018) investigated the protective ability of hBD-MSC on H_2O_2 treated HUVECs [42]. They found that treatment of stressed HUVECs with hBD-MSC or their CM rescued them from H_2O_2-induced endothelial dysfunction (Table 1). In diabetes, the endothelial cells suffer from oxidative stress induced by hyperglycemia leading to several morbidities. In a series of papers, Basmaeil et al. (2020a; 2020b) assessed the ability of hBD-MSC to reverse the damaging effects of high levels of glucose on HUVECs [43,80]. Both hBD-MSC and their CM were found to afford protection to high glucose-treated HUVECs. Although there is much internal inconsistency in the data published in these studies, they suggest that hBD-MSC can protect endothelial cells from oxidative stress. More robust studies are needed to make a conclusive statement.

hPMSC have been evaluated for their anti-inflammatory properties. Particulate matter (PM), a major airway pollutant, causes several adverse effects on health. These particles have been shown to cause inflammation in the middle ear epithelium. Kim et al. [44] used PM-treated human middle ear epithelial cells (HMEECs) as a model system to determine the anti-inflammatory properties of hPMSC (region of the placenta not mentioned). They demonstrated that PM induced a high expression of several inflammatory genes in HMEECs, and these were down-regulated when the PM-exposed-HMEECs were co-cultured with hPMSC [44]. Since the effect was seen in a non-contact culture, it was apparent that it was mediated via secretory factors produced by the hPMSC. Interestingly, increased levels of PGE2 were detected only in the CM of co-cultured hPMSC. Since hPMSC alone did not show such elevated secretion, it was thought that their interaction with the inflamed HMEECs led to increased secretion of PGE2. It is thus possible that priming of hPMSC with inflammatory cytokines could improve their therapeutic application [41]. This aspect was not directly addressed in this study.

Intestinal fibrosis is a severe complication of inflammatory bowel diseases (IBD), including Crohn's disease and ulcerative colitis. Choi et al. (2019) examined the effect of both hUC-MSC and hPMSC (both commercially obtained, region of the placenta used to derive hPMSC not specified) on TGFβ1-treated human primary intestinal myofibroblasts (HIMFs). When the HIMFs were co-cultured with the MSCs using a trans-well insert system, it was observed that both hUC-MSC and hPMSC reduced the TGF-β1-induced fibrosis. This anti-fibrogenic effect was more apparent in the hUC-MSC set [45], showing that despite possessing anti-inflammatory properties, hPMSC were inefficient in reversing fibrosis.

As mentioned earlier, the placenta consists of both fetal and maternal tissues. Papait et al. (2020) used various types of GMP-grade MSCs derived from placenta viz. placenta expanded mesenchymal-like adherent stromal cells (PLX), maternal-derived cells (PLX-PAD), fetal-derived cells (PLX-R18), and amniotic membrane-derived MSCs (hAMSC), and compared their immuno-regulatory properties. They found that though both, fetal and maternal MSCs showed immuno-regulatory activities, the fetal MSCs had a stronger capacity to modulate immune cell

proliferation and differentiation. [24]. These results are consistent with a previous study reported by Kim et al. [46], wherein they had shown that MSCs isolated from the inner side of the chorioamniotic membrane possess immuno-modulatory activity [44].

Khoury et al. (2020) compared the immuno-regulatory activity of amniotic fluid, bone marrow- and chorionic tissue-derived MSCs (hAFSC, hBMSC, hCP-MSC, respectively) using antigen-stimulated leukocytes, lymphocytes, neutrophils, and T cell subsets derived from the blood of healthy donors. The study's principal objective was to determine the equivalence of hCP-MSC to hBMSC, which are commonly used in cell therapy. They found that both, hCP-MSC and hBMSC showed comparable anti-inflammatory effects. While hCP-MSC were found to suppress multiple inflammatory cytokines gene expression through secreted factors, a direct cell-cell contact was needed to affect Th1 cell proliferation. These data suggest that hCP-MSC could be used to target Th1-related diseases [10].

Corneal opacity happening due to various causes is one of the most common corneal disorders, and if left untreated, it could lead to vision impairment. Rose et al. (2020) examined the effect of hPMSC (Maternal origin, cotyledon region) on corneal opacity following experimental injury using organ cultures. Using laser scatter measurements and histo-chemistry, they showed that intra-stromal injection of hPMSC significantly improved the corneal transparency and reduced corneal scarring [47]. This proof-of-principle study suggests the use of hPMSC as an alternative to keratoplasty, which is the standard treatment for corneal opacity.

Dynamic invasion ability of trophoblasts is critically required for successful embryo implantation. Seok et al. (2020) investigated the molecular mechanism involved in the hCP-MSC (Chorionic plate-derived as per their earlier publication)-mediated salutary effect on invasion of trophoblasts derived from human placenta at the early stage of pregnancy. They showed that hCP-MSC improved the invasive ability of trophoblast cells [48]. Their data suggest that hCP-PMSC could be used as a cellular therapy for infertility. Whether the hPMSC derived from a term placenta would also possess such properties needs to be examined, as obtaining early-stage placenta could pose ethical issues.

The use of patient-derived cells, instead of normal cells, as the target population to examine the therapeutic potential of hPMSC would give a better screen to proceed for animal studies. Asthma is a respiratory disease that manifests itself as an allergic and inflammatory condition, wherein Interleukin-5 (IL-5) plays an important role. Lin et al. (2019) examined the effect of human choriodecidual membrane-derived MSCs (hCD-MSC) on peripheral blood mononuclear cells (PBMCs) collected from asthmatic children in a co-culture system. They found that hCD-MSC reduced the activation and proliferation of T cells, and reduced the level of IL-5 in them [49]. Further in-depth studies are needed to take this study to a therapeutic level.

Overall, the data obtained in these studies suggest that hPMSC possesses anti-inflammatory, anti-oxidant, immuno-regulatory, and EC-protective abilities and provides insight into the mechanism of action of hPMSC on the target cells. Although *in vitro* studies form a strong basis to initiate *in vivo* studies in animal models, they do not address the issues related to cell dose, route of administration, safety, etc.

2.2. Animal studies

Therapeutic effects of hPMSC have been assessed using animal models of a plethora of different conditions, which vary from intestinal fibrosis, osteoarthritis, airway pollutants, corneal opacity to infertility, asthma, chronic kidney injury, and dementia. The outcome of these studies would pave the way for clinical trials. Notably, such focussed studies reveal the strengths and weaknesses of hPMSC compared to those of hBMSC and hUC-MSC, which have already entered clinical trials. Some of the studies have been briefly described below and the details are summarised in Table 2.

Ji et al. (2019) compared the effect of hCV-MSC and hBMSC on naphthalene-induced lung injury, wherein they found that owing to their higher expression of CXCL12, hBMSC displayed a significantly more rapid restoration of the airway epithelial cells than hCV-MSC [50]. These data suggest that for this particular application, either hCV-MSC batches would need screening for the levels of CXCL12 present in them, or they may need priming by genetic or pharmacological methods to increase its expression if some of the batches do not show sufficient expression of CXCL12 protein. In other words, the CXCL12 expression level needs to be added as a QA/QC parameter for this particular application. Cargnoni et al. (2020) investigated the protective effect of hAMSC given intratracheally in a mouse model of bleomycin-induced pulmonary fibrosis. Their data confirmed the ability of hAMSC to reduce lung fibrosis by lowering the inflammatory reactions evoked by bleomycin. An important aspect of this study was the use of both, freshly isolated and culture-expanded (passage 2) hAMSC to address the impact of *in vitro* expansion on hAMSC's therapeutic effects [51]. They found that both types of cells had similar effects on the immune populations investigated in this study, suggesting that at least a short-term expansion *in vitro* does not alter cell activity. This aspect is relevant in the clinical application of hPMSC.

Chronic kidney injury eventually leads to end-stage kidney failure. Using Aristolochic acid (AA)-induced chronic kidney failure rat model, Cetinkaya et al. (2019) found that intravenous injections of hAMSC effectively reduced AA-induced inflammation and fibrosis in the kidney. If clinical trials indeed show efficacy, this treatment would be a boon to CKD patients also, as the end-stage CKD patients have dialysis as the only option, and dialysis itself is associated with several complications [57].

Immuno-modulatory effects of hPMSC have been evaluated in the treatment of asthma and graft versus host disease (GvHD) seen in allogenic transplants using specific animal models. Li et al. (2018) infused hPMSC in the ovalbumin (OVA)-sensitised asthmatic rats to examine the role of Notch signaling in the immunomodulatory effect of the infused hPMSC. They found that infusion of hPMSC resulted in a significant reduction of OVA-mediated increase in Notch signaling in the asthmatic rat's lungs. [52]. These results indicate the role of Notch signaling in asthma and show that hPMSC can modulate this pathway.

Hematopoietic stem cell transplantation (HSCT) is the oldest and the most successful stem cell therapy. However, it has its problems like GvHD in an allogenic setting. GvHD leads to morbidity, and even mortality, but some of the patients are refractory to standard-of-care treatments; hence, there is a great need to have alternative approaches. Intravenous infusions of hPMSC have been shown to reduce GVHD-induced morbidity [54,55]. These two studies indicate that hPMSC could help treat patients suffering from GvHD, especially the ones refractory to the routine treatment regimes.

Regeneration of damaged neurons using MSCs is one of the most studied aspects of regenerative medicine. Dementia is a neurodegenerative disease, characterized by denervation of cholinergic neurons in the hippocampus and entorhinal cortex and subsequent memory impairment. A reduction in choline acetyltransferase (ChAT) activity in the hippocampus and frontal cortex is often seen in dementia patients. Using a rat model of dementia, Cho et al. (2018) showed that infusion of hAMSC allowed a significant cognitive recovery; however, this effect was not due to the differentiation of the injected hAMSC into cholinergic neurons, thus showing that the hAMSC possess neuro-regenerative

Table 2
Pre-clinical studies done in murine disease models using human placenta-derived mesenchymal stem cells.

Sr No	Model used	Outcome & critical observations	Dosage & route of administration	Reference
1	Napthalene-induced lung airway injury mouse model	Infusion of Bone marrow-derived MSCs (hBMSC)and human chorionic villi-derived (hCV-MSC) MSCs after 2 weeks showed an increased number of SCGB1A1 (Clara cell secretory protein) positive cells in airways, as compared to control, however, better outcome was seen with (BMSC). Out of the 10 differentially expressed chemokines between the two MSCs, knockdown of only CXCL12 in hBMSCwas done. hBMSC were reported to be better at repair of airway epithelium than hCV-MSCs. This ability of hBMSCs was due to a higher expression of CXCL12 in them, which was confirmed by knockdown of CXCL12.Authors have not discussed if this differential expression of CXCL12 is due to inherent difference between adult and fetal tissue derived MSC.	1×10^6 hCV-MSC were infused via tail vein	50
2	Ovalbumin-induced asthma rat model	The hPMSC transplanted asthmatic rats showed lower levels of inflammatory cytokines than control asthmatic rats. They also exhibited lower expression of *Notch1* and *Notch2*genes. However, it is not clear how hPMSC produced this effect and how long the effect would last. (hPMSCs)	hPMSC 1×10^6 cells per kg were injected intravenously	52
3	192 IgG Saporin –induced dementia rat model	After 35 days, hPMSC transplanted rats showed a reduced number of microglia and showed normalised acetylcholine esterase (AchE) levels in their hippocampus, as compared to their un-transplanted counterparts. Transplanted mice also performed better in maze tests. Effect of hPMSC was comparable to Donepezil.	hPMSC 5×10^6 cells given via intra-venous (IV) and intra-cerebroventricular (ICV) route	53
4	Mouse Graft versus host disease (GvDH) model.	After 14 days, GSH/GSSG ratio improved in mice treated with hPMSC, as compared to those given PBS. hPMSC also reduced the ROS levels in liver and spleen. There was a reduction in the number of PI-1^+ T cells in hPMSC transplanted mice, as compared to PBS control.	1×10^6 hPSMC were infused via tail vein	54
5	Humanised xeno-GvHD mouse model.	After 14 days, hPMSC transplantation led to a reduction of IL6 and IL17 cytokines, a decrease in Th17 cells and an increase in Tr1 cells as compared to untreated controls.	1×10^6 hPMSC were infused via tail vein	55
6	Global cerebral ischemia (GCI) SD rat model.	Administration of hPMSC enhanced the neurogenesis induced by CGI. Contrary to the authors' conclusions, the number NeuN(+) cells in hPMSC transplanted rats do not seem to be different from that seen in control/sham operated rats.	1×10^6 hPMSC injected IV every week till sacrificed.	56
7	Mouse wound healing model	Mesenchymal stem cells were derived from Amnion (hAMSC), blood vessel (hCP-MSC-bv) and Wharton jelly (hUC WJ-MSC). These MSCs were combined or not with placental endothelial cells (hP-EC). Matriderm soaked with these cells was applied to mice wounds and after 8 days, analysis was done. Matriderm combined with MSCs showed faster wound healing and blood vessel formation as compared to that seen in Matriderm alone. No additional advantage of endothelial cells was seen. Effect of only hAMSC, hCP-MSC-bv and hUC- WJ-MSC without Matriderm was not examined.	Matriderm was soaked in 3×10^5 Human PMSCs placed on wound area. Different types of hMSC were mixed or not with placental endothelial cells (80:20 ratio).	8
8	Aristolochic acid (AA)-induced chronic kidney disease (CKD) rat model	hPAMSC were derived from placental Amnion and injected IV into rat CKD model. Western blot showed that PCNA protein levels in hPAMSC transplanted group were lower than that in sham control, while p57 and PARP-1 levels were comparable to sham control. IHC for Collagen -1 and IL-6 showed reduction in hPAMSC transplanted group treated rats compared to positive control. Rats treated with hPAMSC also showed reduction in serum urea, serum creatinine levels compared to AA treated rats.	6×10^5 hPAMSC injected via tail vein	57
9	Bacterial collagen-induced rat Intracerebral hematoma (ICH) model	Human Placenta-derived MSCs (hPMSC) were iinjected 1 h after inducing ICH and their effect was studied after 24 h. Hematoma volume was reduced in hPMSC treated rats. Western blot showed a stable expression of blood brain barrier proteins ZO-1 and occludins in hPMSC treated rats. Rats treated with hPMSC showed reduced death rate following intracerebral haemorrhage.	1×10^6 hPMSC injected via tail vein	58
10	Ovariectomized rat model	The transplanted animals showed slightly higher expression of genes associated with anti-oxidative properties such as catalase, superoxide dismutase and Prdx2; reduced expression of genes associated with apoptosis in the remaining ovary; slightly higher expression of genes associated with generation of new follicles. Overall, hPMSC did not show any negative	5×10^5 PKH dye-labelled hPMSC injected via tail vein. Animals sacrificed at 1,2, 3 and 5 weeks post transplantation. Samples from animals within non transplanted and transplanted animals was pooled	59

Table 2 (*Continued*)

Sr No	Model used	Outcome & critical observations	Dosage & route of administration	Reference
		effects in ovariectomized rats and showed some signs of restoration of ovarian function.		
11	Graves Opthalomopathy (GO) mouse model generated via electroporation of TSHR A-subunit plasmid	Expression of TSHR, TGF β2, ICAM-1 and TNF α decreased in GO mice treated with hPMSC, as compared to sham control, but not significantly different from steroid treated mice. Further, when orbital fibroblasts generated from GO biopsy were co-cultured with hPMSC, the orbital fibroblasts showed reduced expression of adipogenic transcription factors.	3×10^5 hPMSC were injected retro-orbitally	60
12	Pre-clinical toxicity studies for placenta derived decidua stromal cells (DSC)	hDSC were infused at various doses via intra aortal and venous route in mice & rats. The various doses of hDSC infused in mice did not show any adverse effects on weight, lymphocytes, blood cholesterol, triglyceride levels, liver enzymes, but showed increased clotting time. Histopathological analysis of liver and lungs did not show any adverse effect 30 days after hDSC infusion. Thus, hDSC did not cause any adverse reaction upon transplantation.	Human placenta-derived decidua stromal cells (hDSC) given at different doses via intra aorta and intra venous in rats, while were given inter venous in mice	61
13	X-ray-induced local dermal injury in rats	Irradiated rats were injected with either culture medium concentrate (CM), hPMSC L) or concentrate of conditioned medium (CMPL). Complete healing of the open wound surface of the skin in the CM groups was observed in 40 % and the CMPL in 60 % of the treated mice. Newly formed blood vessels increased in the hPMSC group and CMPL groups. The best outcome was detected in the CMPL group. Placenta conditioned medium concentrate (CMPL group) was found to accelerate the transition of the wound process to the stage of regeneration and epithelization.	Human placenta derived mesenchymal stem cells (hPMSC) 2×10^6 cells/kg and 0.4 mL concentrate of conditioned medium was injected intra-dermally.	63
14	Primordial follicle activation in aged female rats	Histological studies of the rat ovaries treated with multiple-injection therapy group showed more than two-fold increase in the number of primary follicles. E2 levels were significantly increased at 5 weeks after multiple-injections of hPMSC, indicating improved ovarian functioning.Through use of computational algorithms, the study provided explanation on how hPMSC transplantation promoted the primordial-to-primary follicle transition - through miRNA altered BMP signaling. Multiple-injections hPMSC therapy were more effective for primordial follicle activation and subsequent follicular development.	5×10^5 hPMSC suspended in 0.2 mL of phosphate-buffered saline (PBS) were injected via the tail vein for a single dose. For multiple-dose therapy, PKH67-labeled hPMSC were injected three times at 10-day intervals or 4-week intervals.	64
15	Bleomycin-induced lung injury in mice	Rabbits injected with hAMSC showed a reduced expression of α-SMA, Fibronectin and Collagen by qRT-PCR suggesting that there would be limited fibrosis in treated rabbit lungs. No appreciable changes were seen in number of macrophages and T cell subsets between control and transplanted groups. Proinflammatory cytokines were lower, while anti-inflammatory cytokines were higher in hAMSC transplanted rabbits compared to controls. hAMSCs reduced fibrosis in lungs by impeding lung B-cell recruitment, retention, and maturation	1×10^6 hAMSC were injected intratracheally.	51

properties [53]. The effects seen with hAMSC were, however, found to be comparable to that shown by Donepezil, which is a commonly used drug to treat dementia. Whether the use of hPMSC helps avoid side-effects of the drug and whether they would work in drug-refractory dementia cases needs to be examined in clinical trials so that the higher cost associated with cell therapy can be justified.

Transient neurogenesis followed by an anergic state is a hallmark of global cerebral ischemia (GCI) and poses a significant obstacle for cardiac arrest survival. Kho et al. (2018) found that IV infusions of hCP-MSC to the rats transiently exposed to GCI resulted in a significantly increased count of NeuN positive cells in their hippocampi 4 weeks after GCI [56]. However, it is not clear how intravenously administered hCP-MSC crossed the blood-brain barrier to reach the hippocampus and specifically expressed ZnT3 to induce endogenous neurogenesis. The molecular mechanism involved in the increased ZnT3 expression needs to be elucidated to confirm this initial result.

Non-healing wounds or ulcers pose a massive challenge in managing diseases like diabetes, as these patients bear a high risk of infections leading to sepsis and amputations. Ertl et al. (2018) applied hAMSC, hCP-MSC, and hUC-WJC topically, with or without co-application of placental endothelial cells, using Matriderm as a carrier onto skin wounds in mice. They found that all three types of MSCs induced a significantly faster wound healing coupled with neo-vascularization compared to Matriderm alone [8]. Co-application of placental endothelial cells did not confer any additional advantage over the application of MSCs alone. Since Matriderm itself has substantial wound healing ability, the use of an inert carrier would have revealed the true wound healing potential of the cells applied.

In addition to the studies mentioned above, hPMSC have been used to treat ICH [58], ovarian dysfunction [59], Graves'

ophthalmopathy (GO) [60] as well as radiation injury [62,63], and the results are very encouraging (Table 2). Pre-clinical toxicity studies of hPMSC are a prerequisite for taking them to clinical trials. Sadeghi et al. (2019) performed such studies with human placenta-derived decidua stromal cells (hDMSC). They found that the hDMSC are safe with almost no side effects even with doses 40 times higher than those used clinically. However, hDMSC showed a higher propensity for clotting, and therefore, supplementation with low-dose heparin was needed. They also found that intra-arterial route of infusion leads to the distribution of hDMSC into abdominal organs instead of lungs, as is seen with IV infusions [61]. This approach would avoid pulmonary trapping of cells but, importantly, would be also helpful when the target organs other than lungs are involved. Such pre-clinical studies should be done with MSCs isolated from all parts of the placenta so that their safety can be assessed and their clinical application can be expedited.

Overall these studies suggest that one cannot blindly apply hPMSC for various conditions. Comparative studies using MSCs isolated from various parts of the placenta need to be done, and also their equivalence or superiority with hBMSC and hUC-MSC for a particular application needs to be established. Nonetheless, these studies underscore an important point that hPMSC could offer an additional option for patients not responding to routinely applied drugs.

3. Priming of PMSCs to improve their therapeutic effects

Cultured MSCs are known to lose their potency, and hence, they may need priming with pharmacological agents or genetic manipulations to improve their therapeutic effects [41]. Several studies have used this approach with hPMSC and these have been reviewed here. These are summarised in Table 3.

One of the most common approaches to improve the functionality of hPMSC is to culture them in 3D matrices so that they regain their *in vivo* properties [65]. 3D spheroid cultures of hAMSC have been shown to possess better angiogenic and immunosuppressive properties, than their 2D counterparts [66–68]. Likewise, 3D spheroids of hCP-MSC were shown to have significantly higher restorative effects on ovarian functions in a rat model of premature ovarian failure (POF) [68]. This approach would help treat menopausal complications and avoid the use of hormonal therapy, which is associated with a risk of developing breast cancer. Data from a series of papers on hPMSC cultured on chitosan (CS) hydrogel with immobilised IGF-C (C domain peptide of insulin-like growth factor 1) show that CS-IGF-C hydrogel significantly improves their therapeutic effects in various disease models of hind limb ischemia, colitis, acute myocardial infarction and acute kidney injury [69–72]. Similar results have also been reported with hPMSC grown in self-assembled GFFYK peptide hydrogel and nitric oxide releasing hydrogel for mouse hind limb ischemia [73,74]. These effects could be attributed to better retention of cells at the site and increased angiogenesis.

GvHD and colitis are associated with increased levels of inflammatory cytokines like IL-17, IL-1β, and IFN-γ. Interestingly, priming of hPMSC with these very factors implicated in the disease pathology has been shown to confer better immuno-modulatory activity on them, and consequently, these primed hPMSC exerted better therapeutic effects in the respective experimental models [75–,76,77].

Min et al. used Apocynin, a well-known inhibitor of NADPH oxidase, to prime the hPMSC and examined their effect on rats suffering from bacterial collagenase-induced intracerebral haemorrhage (ICH) [78]. They found that primed hPMSC significantly reduced the hematoma size, hemispheric enlargement, and destruction of neurons, coupled with improved microvascular integrity in the brains of ICH rats.

Many times, the infused stem cells face an oxidative environment, where they need to survive to induce therapeutic effects. To improve their survival and functionality in such a stressful environment, priming of hPMSC with oxidative stress inducers like H_2O_2 or glucose was tried with success [79,80]. Angiogenesis is one of the essential pre-requisites for efficient wound healing. Matthew and Bhonde (2018) showed that Polyunsaturated fatty acids (PUFAs) treated chorionic villous region-derived hPMSC (hCV-MSC) exhibited better angiogenic properties [81]. Jagged1 (JAG1), a Notch ligand, is abundantly expressed and markedly increased in the cartilage of osteoarthritis (OA) patients [82]. Consistent with this report, inhibition of Notch signalling in chondrocytes has been shown to suppress OA in mice [83]. Based on these reports, Sun et al. (2018) examined whether soluble Jagged-1, an inhibitor of Notch signalling, enhances the chondrogenic potential of hCV-MSC. They found that injections of soluble Jagged-1-primed hCV-MSC in the knee joint of mice experimentally induced with post-traumatic OA resulted in enhanced cartilage formation and decreased joint inflammation [84].

The effect of genetically modified hPMSC to enhance their therapeutic effects towards particular application has been investigated by many researchers; however, application of genetically modified hPMSC would evoke additional safety concerns.

Abnormal vascularization has been implicated in preeclampsia, and hence, improving placental angiogenesis might be an attractive approach to treat this condition. Heme oxygenase-1 (HO-1) plays a key role in angiogenic factor expression during pregnancy, and therefore, Wu et al. (2020) examined whether the use of HO- modified hPMSC would have an additional advantage in promoting angiogenesis. They found that HO-1 reduced the apoptosis of hPMSC (region of the placenta not specified) and increased VEGF expression in them. Conditioned medium of HO-1-modified hPMSC exhibited better angiogenic properties as assessed by matrigel tube formation assay and villus-decidua co-culture system [85]. However, the use of villus-decidua from the placenta collected from women suffering from preeclampsia could have made the study more robust.

Hemophilia A (HA) is an X-linked recessive disorder caused by mutations in the Factor VIII (FVIII) gene leading to deficient blood coagulation. Using a murine neonatal HA model Gao et al. (2019) sought to determine whether co-transplantation of endothelial colony-forming cells (ECFCs) and hCV-MSC (First-semester placenta, early gestation chorionic villi-derived stromal cells) modified with factor VIII could achieve long-term engraftment and FVIII expression. The tail clip assay resuts showed that the blood loss volume in HA mice receiving co-transplantation was significantly less, than controls, indicating significant attenuation of bleeding symptoms [86]. Although the data are encouraging, compared to mice, human beings have a long life-span, and hence, issues like how long the infused hCV-MSC survive in the recipients' body and whether a single infusion would suffice to provide a life-long supply of factor VIII needs to be examined in clinical trials.

During hepatic regeneration, PRL-1(phosphatase of regenerating liver-1), an immediate early gene, promotes vascularization by increasing portal flow. Kim et al. [44] showed that PRL-1-modified hCP-PMSC showed a higher vascular remodeling and hepatocyte proliferation in a rat BDL (hepatic failure induced by bile duct ligation) model [87]. However, liver function data need to be generated to shift to a translational model.

Pigment Epithelium-derived factor (PEDF) plays a protective role in retinal pigment epithelial (RPE) cells from oxidative stress. Kim et al. [64] co-cultured PEDF-modified MSCs isolated from the inner side of chorioamniotic membrane (hACM-MSC as per their

Table 3
Studies involving the use of primed human placenta derived mesenchymal stromal cells and extra cellular vesicles secreted by them.

Sr No	Intervention /Condition/experimental system	Outcome & critical observations	Dosage & route of administration	References
1	Comparative paracrine functioning of human amnion-derived mesenchymal stromal cells (hAMSC) cultured in 2D and 3D culture systems	3D culture conditions effectively induced hAMSC spheroid formation, maintained multi-potency of the cells and improved their paracrine activity. 3D-hAMSC displayed enhanced paracrine induction, cell migration and tubulogenesis of human umbilical vein endothelial cells (HUVEC). Angiogenic growth factor- and immunosuppressive factor-related genes were significantly upregulated in 3D-hAMSCs when compared to 2D-hAMSC. 3D hAMSC exhibited significantly greater expression levels of pluripotent markers OCT4, SOX2 and NANOG indicating higher viability and multipotency in 3D culture.	*In vitro* study	66
2	Comparative study of the production of soluble factors in human amniotic membrane of term placenta-derived mesenchymal stromal cells (hAMSC) grown in adherent conditions or in semipermeable and biocompatible fibre	hAMSC were cultured for 24 and 48 h as monolayers or were encapsulated in a catheter-like device.Secretion of SDF1α, Gro-α, IL-6, IL-8, and MCP-1 was significantly enhanced in encapsulated hAMSC, as compared with the monolayer culture.hAMSC aggregated in the device constituted an effective system for enhancing, or at least for maintaining, the paracrine activity of these cells in order to better promote tissue regeneration in an immune isolated state.	*In vitro* study	67
3	Study the therapeutic effect of placenta-derived mesenchymal stem cells (hPMSC) spheroids in restoring ovarian functions and compare their efficacy to 2D cultured hPMSC	Spheroidal 3D- and 2D-cultured hPMSC were engrafted into ovariectomized (Ovx) rats and their beneficial effects were compared.Serum levels of oestradiol hormone were significantly higher in the spheroid group at 2 weeks post transplant. Spheroid hPMSC also exhibited a significantly higher efficiency of engraftment into the ovarian tissues at 2 weeks. Transplantation of spheroid hPMSC also promoted expression of folliculogenesis-related genes in the Ovx rats. Nanos3, Nobox, and Lhx8 were significantly upregulated at the mRNA and protein levels in the spheroid group compared with those in the non-transplantation (NTx) group at 1 and 2 weeks. The number of follicles also increased in rats treated with hPMSC spheroids at 1 week.Overall spheroid hPMSC had a higher therapeutic effect than naive 2D hPMSC in restoring ovarian functions.	100 hPMSC spheroids or 1×10^5 naïve hPMSCs were directly transplanted into the remaining ovary one week after ovariectomy	68
4	Investigate the effect of co-transplantation of a chitosan (CS)-based injectable hydrogel with immobilized IGF-1 C domain peptide (CS-IGF-1C) and human placenta-derived MSC (hPMSC) on ameliorate colitis	The CS-IGF-1C hydrogel significantly increased hPMSC engraftment, proliferation and promoted the production of PGE_2 from hPMSC *in vitro*. *In vivo* studies indicated that the CS-IGF-1C hydrogel promoted hPMSC survival and markedly alleviated mouse colitis, which was possibly mediated by PGE_2 secreted by hPMSC and interleukin-10 (IL-10) produced by polarized M2 macrophages.	1×10^6 hPMSC suspended either in PBS, CS hydrogel, or CS-IGF-1C hydrogel were injected at three sites of the injured colon mesangial margin through the connection between the mesentery and intestinal wall	70
5	Investigate the effect of CS-IGF-1C hydrogel on the survival and efficacy of human placenta-derived mesenchymal stem cells (hPMSC) in the treatment of acute myocardial infarction (AMI)	hPMSC were transplanted with insulin-like growth factor-1 on chitosan (CS-IGF-1C) hydrogel into a mouse myocardial infarction model.CS-IGF-1C hydrogels induced the proliferation of hPMSC and improved their survival by exerting an anti-apoptotic effects *in vitro*. hPMSC seeded on CS-IGF-1C hydrogel protected neonatal mouse ventricular cardiomyocytes against oxidative stress. Histology analyses indicated increased angiogenesis, reduced collagen deposition, and expansion of left ventricle, that promoted the recovery of cardiac function. Inflammatory responses were also inhibited and the expression of apoptosis-related genes were downregulated by hPMSC co-transplanted with CS-IGF-1C hydrogel.	5×10^5 hPMSC were added to 20 μL CS-IGF-1C hydrogel and injected at two positions adjacent to the infarcted areas	71
6	Design and synthesis of a functional hydrogel-based scaffolds to enhance the therapeutic efficacy of human placenta-derived mesenchymal stromal cells (hPMSC) in a murine acute kidney injury (AKI) model	Self-assembling naphthalene (Nap) covalently linked to a short D-form peptide (Nap-DFDFG) and the C domain of insulin-like growth factor-1 (IGF-1C) formed a hydrogel-niche for hPMSC. IGF-1C hydrogel significantly improved hPMSC	2×10^6 hPMSC were intra-renally injected at two random sites of right kidney cortex at 40 μL total volume suspended in β-IGF-1C hydrogel respectively	72

Table 3 (*Continued*)

Sr No	Intervention /Condition/experimental system	Outcome & critical observations	Dosage & route of administration	References
		retention at the site of injection. Co-transplantation decreased expression of injury markers while increased expression of regeneration markers, alleviated deposition of collagen fibres, amplified the VEGF/VEGFR2 pathway, and promoted angiogenesis.		
7	Design and synthesis of a bioactive and biocompatible hydrogel, Nap-GFFYK-Thiol to enhance the retention and engraftment of human placenta-derived MSC (hPMSC) in a mouse ischemic hind limb model	Nap-GFFYK-Thiol hydrogel enhanced the proangiogenic and anti-apoptotic effects of hPMSC. *In vivo*, the hydrogel enhanced hPMSC retention at site of injection, improved blood perfusion leading to superior limb salvage, suppressed collagen deposition, increased the proangiogenic properties of hPMSC and further promoted the angiogenesis of autologous vascular cells in ischemic mouse hind limbs. The hydrogel serves as an artificial niche for promoting hPMSC survival and proangiogenic factor secretion.	3×10^7 hPMSC suspended in 100 μL of hydrogel were immediately injected into three different sites in the right adductor muscle adjacent to and within 1 mm proximal or distal to the ligation site	73
8	Design of hydrogel that releases nitric oxide to enhance effect of mesenchymal stem cells for treating hind limb ischemia in mice	The placental mesenchymal stem cells cultured on Chitosan-Nitric oxide (CS-NO) hydrogels showed good cell viability and no apoptosis. Placental MSC (hPMSC) grown in CS-NO hydrogels when transplanted into ischemic limbs showed low ischemic damage and impairment than that seen in animals receiving PMSC alone. PMSCs on CS-NO hydrogels were able to restore blood supply in the ischemic limb. Masson's trichrome staining showed increased muscle regeneration in PMSC CS-NO hydrogel treated ischemic hind limbs.	1×10^6 cells (hPMSC) were injected at 3 sites of adductor muscle and gastrocnemius muscle in ischemic hind limbs. The cells were suspended in either PBS, chitosan hydrogels or chitosan-nitric oxide hydrogels. The injected animals were studied after 4 weeks.	74
9	Investigate the paracrine role of placental mesenchymal stromal cells (hPMSCs) in three-dimensional culture of colon with experimental colitis	hPMSC were primed with 50 ng/ml interferon (IFN)-y) to enhance their immunomodulatory effects. Acute colitis was induced in mice by oral administration of 2% dextran sulfate sodium (DSS). Colon explants from mice administered with DSS were treated with hPMSC, conditioned media (CM) or DMEM for 24 h.hPMSC and CM-treated colons from DSS mice revealed fewer inflammatory infiltrates, lesser extent of inflammation and lower crypt structure damage, as compared to DMEM-treated group. CM treatment increased cell proliferation and hPMSC treatment reduced $CD3^+$ cells in colon tissue, whereas hPMSC treatment showed no effect on cell proliferation. Levels of pro-inflammatory cytokine IL-6 were elevated in supernatant of untreated colitis group compared with the naïve group. hPMSC and CM treatments down-regulated IL-6 levels to values nearly similar to those in healthy colonic explants. Data show that hPMSC and CM treatments can alleviate colonic damage in organ culture.	Colon tissues from DSS fed male C57BL/6 mice were treated for 24 h with hPMSC (1×10^5 cells/200 μL DMEM) or CM (200 μL) into 96-well dish under submerged conditions	75
10	Investigate the effects of IL-27 on human placenta–derived MSCs (hPMSC) to induce generation of CD4+IL-10+IFN-γ+ T cells *in vitro* and in the humanized xenogenic GvHD NOD/SCID model	The percentages of $CD4^+IL\text{-}10^+IFN\text{-}g^+$ T cells significantly increased in activated human PBMC obtained from both healthy donors and GvHD patients and also in the liver and spleen of hPMSC-treated GvHD mice. Frequency of $CD4^+IL\text{-}10^+IFN\text{-}\gamma^+$ T cells in the liver was greater than that in the spleen in hPMSC-treated GvHD mice. Serum level of IL-27 decreased and the symptoms abated in hPMSC-treated GvHD mice. IL-27 promoted the regulatory effects of hPMSCs by enhancing the generation of $CD4^+IL\text{-}10^+IFN\gamma^+$ T cells from activated PBMC. Activation occurred through increases in the expression of programmed death ligand 2 (PDL2) via the JAK/STAT signaling pathway. hPMSC could alleviate GvHD mice symptoms by upregulating the production of $CD4^+IL\text{-}10^+IFN\gamma^+$ T cells in the spleen, liver, and downregulating serum levels of IL-27.	1×10^6 hPMSC injected via the tail vein	76
11	Analyse the role of IL-1β on immunomodulatory effects of human placenta-derived mesenchymal stromal cells (hPMSC) on graft-versus-host disease (GvHD) model	IL-1β-primed hPMSCs decreased IL-1β levels, and downregulated Th1/Th2 and Th1/$CD4^+IL\text{-}10^+$ T cell ratios in the GvHD model. The results revealed that IL-1β strengthened the hPMSC	1×10^6 hPMSC were injected intravenously and mice were sacrificed on day 7 and day 14 post-transplant	77

Table 3 (*Continued*)

Sr No	Intervention /Condition/experimental system	Outcome & critical observations	Dosage & route of administration	References
		capacity to reduce the Th1/Th2 and Th1/$CD4^{+}IL\text{-}10^{+}$ T cell ratios, inhibited the adhesion and proliferation of hPMSC and increased PD-L1 expression on hPMSCs via the JAK and NF-κB pathways.hPMSC alleviated GvHD by decreasing IL-1β level and maintaining the balance among different T cell subsets. IL-1β in turn enhanced the ability of hPMSCs to balance different T cell subsets and inhibited hPMSC adhesion and proliferation by regulating PD-L1 expression via the JAK and NF-κB pathways.		
12	Effect of preconditioning of human placenta-derived mesenchymal stem cells (hPMSC) with nicotinamide adenine dinucleotide phosphate (NADPH) oxidase inhibitor on the therapeutic efficacy of hPMSC in intracerebral haemorrhage (ICH)	Apocynin-pre-conditioned human placenta-derived MSCs (Apo-hPMSC) when injected in ICH mouse model showed a significant reduction in hematoma size, hemispheric enlargement and degenerating neuron count compared to the naïve hPMSC group. Expression of tight junction protein, occludin, was higher when mice were treated with Apo-hPMSC suggesting that primed cells are more efficient in blocking leakage of blood components from ruptured vessels to brain parenchyma after ICH than naïve hPMSC.	1×10^{6} apocynin-preconditioned hPMSC	78
13	Study the effect of mesenchymal stromal cells from the decidua basalis (hBD-MSC) preconditioning to a stress environment (exposure to hydrogen peroxide)	hBD-MSC preconditioned with H_2O_2 showed enhanced proliferation, colonogenicity, adhesion, and migration by increasing the expression of pro-survival genes and decreasing the expression of several oxidative stress-related genes in a dose-dependent manner.	*In vitro* study	79
14	Evaluate the beneficial effects of glucose on mesenchymal stromal cells from the decidua basalis (hBD-MSC) functions	hBD-MSC were exposed to high levels of glucose and its effect on cell phenotype, functional properties and production of oxidative stress and immunomodulatory molecules was analysed.Conditioning of hBD-MSC with glucose improved their adhesion and invasion, increased expression of genes related to proliferation, migration, invasion, anti-inflammatory, anti-chemoattractant and antimicrobial properties. Interestingly, glucose modulated hBD-MSC expression of genes, which are involved in insulin secretion, and prevention of diabetes. Preconditioning of hBD-MSC with glucose enhanced their therapeutic potential.	*In vitro* study	80
15	Study effect of omega-3 (N-3) polyunsaturated fatty acids (PUFAs) on the angiogenic potential of mesenchymal stromal cells derived from the placenta (hPMSC)	PMSCs were exposed to a mixture of Docosahexaenoic acid (DHA) and Eicosapentaenoic acid (EPA) at different concentrations. Expression of bFGF, VEGF and VEGFR2 increased on priming of cells with PUFA. The treatment also enhanced *in vitro* tube formation ability of hPMSC. Treatment of HUVEC with the conditioned media (CM) of PMSCs enhanced angiogenesis. These results were validated in an *in vivo* matrigel plug assay that revealed enhanced migration and vessel formation in CM treated with DHA: EPA. Supplementation with lower concentration of PUFA enhances the angiogenic potential of hPMSC.	*In vitro* study	81
16	Determine if Notch inhibition could accelerate placenta-derived mesenchymal stromal cells (hPMSC)-induced cartilage regeneration	Placenta-derived mesenchymal stromal cells (hPMSC) were treated with 10 μg/mL JAG1 to inhibit Notch signalling and transplanted in mouse post-traumatic osteoarthritic (PTOA) knee joints. hPMSC pre-treated with soluble JAG1 significantly enhanced chondrogenesis. JAG1-treated hPMSC induced cartilage repair, enhanced cartilage formation, reduced cartilage degeneration, inhibited chondrocyte apoptosis, and decreased joint inflammation at 4 weeks after injection. JAG1/hPMSC induced thicker cartilage formation than control hPMSCs. sJAG1 was found to induce more cartilage matrix formation when combined with hPMSC.	8 μl PBS solution containing 2×10^{5} hPMSC mixed with sJAG1 (10 μg/mL) or lgG (10 μg/mL) were injected once a week for 4 weeks into the joint cavity from the medial edge of the patellar ligament in each group	84
17	Analyse effects of heme oxygenase-1 (HO-1) overexpression on placental vascularization using human placenta-derived mesenchymal stem cells (hPMSC)	HO-1 significantly improved the proliferation and migration of hPMSC while reducing hPMSC apoptosis. Levels of angiogenesis factor VEGF were increased in HO-1-hPMSC, whereas those	*In vitro* study	85

Table 3 (*Continued*)

Sr No	Intervention /Condition/experimental system	Outcome & critical observations	Dosage & route of administration	References
		of anti-angiogenesis factor sFlt-1 decreased. HO-1-hPMSC promoted HUVEC proliferation, migration, and tube formation. When cultured with HO-1-hPMSC-conditioned media, HUVEC branch points increased compared to those in the hPMSC group. HO-1-hPMSC treatment also displayed more advanced remodeling and deeper invasion in the villus and decidua co-culture system. The villus-decidua co-culture system confirmed that HO-1-hPMSC are conducive to angiogenesis and placental vascular remodeling. HO-1-modified hPMSC improve placental vascularization by promoting a balance of pro- and anti- angiogenesis factors.		
18	To determine whether co-transplantation of VIII-expressing endothelial colony-forming cells (ECFC) and placenta-derived mesenchymal stromal cells (hPMSC) can achieve long-term engraftment and Factor VIII expression	ECFCs and hPMSC were transduced with a B domain deleted factor VIII (BDD-FVIII) expressing lentiviral vector. Cells were transplanted intramuscularly into neonatal or adult immunodeficient F8 knock-out HA mice. ECFC only and the co-transplantation groups, but not the hPMSC only, group achieved a long-term engraftment for at least 26 weeks, and the co-transplantation group showed a higher engraftment than the ECFC only group at 16 and 20 weeks post-transplantation. Engrafted ECFCs expressed FVIII, maintained endothelial phenotype, and generated functional vasculature. Co-transplantation also significantly reduced the blood volume loss and attenuated the bleeding symptom. hPMSC significantly enhanced ECFCs engraftment at the later time points of post-transplantation.	3×10^5 ECFCs and 2×10^5 hPMSC (both genetically modified with VIII-expression vector) were resuspended in 16.7 μl ECGM-MV2 media and 3.3 μl of Matrigel and injected intramuscularly into the left hind limb, 3×10^5 hPMSC were injected for the hPMSC only group. Cells were transplanted to mice at 3–5 days of age (neonatal group) and at 12 weeks of age (adult group).	86
19	Analyse the expression of miRNAs and investigate their therapeutic mechanisms for hepatic regeneration via phosphatase of regenerating liver-1 overexpression PRL-1(+) in a rat model with bile duct ligation	PRL-1(+) hPMSC had increased migration ability under hypoxic conditions compared with normoxic conditions. Migrated PRL-1(+) hPMSC displayed improved integrin-dependent migration ability through Ras homolog (RHO) family-targeted miRNA expression. Moreover, miRNA regulated engraftment into injured rat liver by transplanted PRL-1(+) hPMSC through the integrin family. PRL-1(+) hPMSC improved vasculature in liver by enhancing platelet-derived growth factor receptor A. Decreased miRNA correlated with increased proliferation of hepatocytes in liver tissues by activating the interleukin-6 signaling pathway. PRL-1 (+) hPMSC demonstrated regenerative effects on injured liver.	Intravenously transplanted into the tail vein	87

previous report; cultured with FGF4) with H_2O_2-treated RPE cells (ATCC ARPE-19). PEDF-modified hACM-MSC promoted mitochondrial respiration in stressed RPE cells. Similar results were also observed in the H_2O_2-induced retinal degeneration rat model [88]. These data indicate that the PEDF-modified hACM-MSC could form a new treatment for retinal degeneration diseases.

Collectively, these studies underscore the importance of understanding the molecular mechanisms involved in the disease's pathogenesis so that the hPMSC can be appropriately primed to improve their therapeutic potential. As mentioned before, *in vitro* studies need to be validated using appropriate animal models, which could form the basis of clinical trials.

4. Human studies

Clinical trials are the ultimate proof of any experimental therapy's utility; cellular therapy is no exception to this. Not many studies pass the pre-clinical phases due to various reasons, and hence, the results of ongoing clinical trials are eagerly awaited. Efforts to determine the therapeutic potential of hPMSC are going on (https://clinicaltrials.gov/) [89]. During the review period, four clinical studies have been published (Table 4). The outcome of these studies is encouraging and calls for larger studies to determine the efficacy conclusively. However, as mentioned earlier the region of placenta used for the generation of MSCs needs to be specified and the culture conditions need to be critically documented in every study so that data obtained in various labs can be compared.

hDMSC (maternal origin) express programmed death-ligand 1 (PD-L1), PD-L2, and CD49d (a marker of homing to inflamed tissue) at a much higher level, as compared to hBMSC, and hence, are suitable for the treatment of GvHD. Ringden et al. (2018) carried out a pilot study in which they treated 38 patients having acute GvHD using two protocols (Table 3). Importantly, out of these 38 patients, 25 patients were steroid-refractory. They found that the overall severity of GvHD and mortality due to GvHD was significantly less in patients given multiples doses of hDMSC, even in the steroid-refractory patients. Recently, they also performed a pilot study using hDMSC in 6 pediatric patients suffering from steroid-refractory acute GvHD after stem cell

transplant [90]. Five-year survival was at 67 % and all survivors showed a Lansky score of 100 %. None of the children died of GvHD. [91]. These promising results form a strong basis for a larger prospective trial.

In a double-blind, placebo-controlled clinical study, Soltani et al. (2018) evaluated the effect of intra-articular injection of hPMSC (Placenta region not specified) in OA patients. Patients receiving hPMSC showed a significant improvement in knee flexion range of movement (ROM) and reduced pain. Till 8-weeks post-treatment, a significant improvement in quality of life, daily as well as sports/recreational activities, and a reduction in OA symptoms were also seen. At 24 weeks, however, these changes were not significant, though chondral thickness was improved in about 10 % of the total knee joint area in the patients receiving cells [92]. A large-scale study will be required to determine efficacy.

Wang et al. (2019) injected hPMSC (whole placenta used to derive PMSCs) to treat diabetic foot in 4 patients. After 24 weeks, the resting pain and limb coldness scores significantly decreased, and pain-free walking distance significantly increased from baseline. The magnetic resonance angiography findings showed an increase of collateral vessel formation only in one patient [93]. These results, though encouraging, are too preliminary, and a larger trial using placebo control needs to be done.

However modest may be the outcome, clinical trials are the most crucial aspect of any new therapy. The leads obtained in the pilot studies need to be rigorously pursued by doing robust well-designed double-blind large-scale trials to take cellular therapy closer to becoming standard-of-care treatments.

5. PMSC-EVs

Extra-cellular vesicles (EVs) form one of the most important mode of intercellular communication. Initially, the therapeutic effects of MSCs were considered to be due to their tissue-specific differentiation and integration into the damaged tissue. However, as the understanding of the regenerative process increased, it became evident that MSCs exert their effects through the secretion of paracrine factors, collectively called secretome: EVs form an important constituent of this secretome [94,95]. The role of placental EVs in placentation and pregnancy disorders has been reviewed recently [96,97], and hence, not reiterated here.

EVs are broadly classified into three subtypes based on their size and mode of genesis viz. apoptotic bodies, micro-vesicles (MVs), and exosomes [98]. Briefly, apoptotic bodies are secreted by apoptotic cells and usually exert detrimental effects on the target cells, and hence, are not relevant in the context of regenerative medicine. MVs are shed directly from the plasma membrane through multiple mechanisms, whereas exosomes are secreted via the multi-vesicular endosomal pathway. However, once secreted in the surrounding medium their mode of action on the target cells remains the same, though the effects could be vastly different owing to the difference in their contents. Both MVs and exosomes carry a cargo of various macromolecules like mRNA, miRNA, proteins, metabolites, etc, and this molecular profile critically depends on the type as well as the biochemical status of the parent cells and mimic the effects of the parent cells [99–101,41].

Here we review publications describing various applications of hPMSC-derived EVs in regenerative medicine. Most of the papers use the terms EVs, MVs, and exosomes in an interchangeable manner, and therefore, the precise role of MVs vs. exosomes does not become apparent. Our work shows that the macromolecular profile of these two entities differs considerably, and hence, they should be studied independently [100]. In this review we have used the term as mentioned in the cited paper.

Notably, most of the studies published during this period deal with both, hPMSC and their EVs, and most of them have done both *in vitro* as well as *in vivo* studies giving a better insight into the mechanistic aspects. Therefore, these studies have been discussed together. The wide range of disorders being investigated for the possible therapeutic applications of hPMSC-EVs indicates that this field is making rapid progress.

Duchenne muscular dystrophy (DMD) is a debilitating degenerative disease of skeletal and cardiac muscles caused by mutations in the dystrophin gene. hPMSC and the exosomes derived from them have been shown to have therapeutic effects on DMD [102]. They showed that treatment of DMD myoblasts with exosomes isolated from hPMSC (commercial source, region of the placenta not defined) improved their differentiation, reduced the expression of fibrogenic genes, and increased the expression of utrophin, which is an autosomal homolog of dystrophin and can substitute for dystrophin's functions, including assembly into a large transmembrane glycoprotein complex and binding to actin filaments. These effects could be partly attributed to the presence of miR-29c in the exosomes. Consistent with these *in vitro* results, they found that intramuscular transplantation of hPMSC in mdx mice decreased the expression of TGF-β and the level of fibrosis in the diaphragm and cardiac muscles, inhibited inflammation, and increased utrophin expression. Using *in vivo* image analysis, they showed that the transplanted cells could be detected in the muscle tissues up to 3 weeks post-treatment. However, it is important to examine whether miR-29c is consistently found in the exosomes of independent isolates of hPMSC. Also, whether the hPMSC derived from various sites of the placenta have this miRNA in their exosomes needs to be examined.

Kim et al. [46] used a rodent model of Parkinson's disease. hAMSC and hAMSC-derived neural phenotype cells (hAMSC-NPC). (Full-term placenta- amniotic MSCs with ectodermal lineage characters were used). They found that the 6-OHDA-treated rats receiving hAMSC-NPC transplantation exhibited a longer-lasting recovery in motor deficits, than their counterparts receiving saline or hAMSC. *in vitro* studies showed that hAMSC-NPCs efficiently protected primary neural precursor cells from the midbrain against 6−OHDA, and induced their differentiation into DA neurons [103]. They found that hAMSC-NPC conditioned media recapitulated this effect. Whether EVs mediated the effect of CM was not determined. Since the effect of hAMSC-NPCs was better than that of hAMSC, it appears that priming of hAMSC with neurotrophic factors used in their differentiation boosted their therapeutic potential.

Pishavar et al. (2020) evaluated the osteogenic capability of hPMSC-derived EVs (region of the placenta not specified) and compared them to the hBMSC-derived EVs. They found that 64 miRNAs related to osteogenesis were expressed at a higher level in hPMSC-EVs, than hBMSC-EVs. *in vitro* osteogenic differentiation study revealed that the EVs isolated from osteogenically differentiated hPMSC and hBMSC stimulated osteogenesis from naïve hBMSC [9]. The data suggested that EVs from osteogenically primed hPMSC as well as hBMSC have better bone regenerative ability.

Hao et al. [104] found that EVs secreted by hCV-MSC express integrin α4β1 and could improve endothelial cell (EC) migration and vascular sprouting in an *ex vivo* rat aortic ring assay. They further showed that EV-modified electrospun scaffolds significantly promoted EC survival and boosted the expression of angiogenic genes in them [104]. It should be noted here that the hCV-MSC used in this study were routinely cultured in a medium supplemented with bFGF and EGF, and thus, the properties of EVs isolated from such "growth factor-primed" hCV-MSC could be different from those isolated from naïve hCV-MSC. This aspect needs to be addressed.

Tao et al. (2019) investigated whether hPMSC-EVs (region of the placenta not specified) could ameliorate cornea's alkali injury in the mouse model. They found that the hPMSC-EVs increase the

corneal epithelial cell migration in vitro and also exerted a salutary effect on corneal wound healing by inhibiting angiogenesis and inflammation *in vivo*. This effect was seen after a repeated injection of 100 μg of EVs (given in three split doses daily, for 2 weeks). Inhibition of angiogenesis by hPMSC-EVs is an intriguing finding, as these EVs are known to possess pro-angiogenic effects [105]. This apparently contradictory aspect needs to be examined.

Bio-distribution of EVs is an important aspect to be considered for their ultimate clinical application. The infused EVs need to home into the target tissues to achieve the desired regenerative effect. Since EVs are nanosize particles, tracking them *in vivo* poses problems as dyes like PKH-26 or Dil could aggregate in solution giving erroneous results. Cao et al. (2019) used various fluorescent dyes to label the hPMSC-derived EVs and infused them intravenously in a mouse model of acute liver failure (region of the placenta not specified) [107]. Although one of the study's main objectives was to compare the *in vivo* tracking efficiency of various dyes, they also assessed EVs' regenerative potential. They found that infusion of EVs resulted in a reduced expression of genes related to inflammation and apoptosis in the liver tissue. Enhanced proliferation of liver cells and lowering of serum ALT and AST were also evident. The histopathology examination revealed that the liver of EV-treated mice had near-normal pathology and there was no necrosis. Liver fibrosis was also significantly reduced.

Sox-9$^+$ progenitor cells possess a high proliferative ability and contribute to renal regeneration after acute kidney injury (AKI). Using high-resolution intravital *in situ* imaging techniques, Zhang et al. (2020) examined the kinetics of Sox9$^+$ cell proliferation in mouse AKI treated with hPMSC-EVs (region of the placenta not specified). They found that intravenous infusion of hPMSC-EVs reduced apoptosis in kidney cells, promoted proliferation and activation of Sox9$^+$ cells and accelerated renal regeneration. They found that Sox9 was mainly expressed in proximal tubule epithelial cells with evident epithelial polarity. Importantly, Sox-9$^+$ cells in EV-treated mice showed the formation of functional renal tubes. They further found that EVs significantly reduced the post-injury fibrosis in the kidney as seen by histological observations and confirmed by a reduced expression of *fibronectin* 1 (*Fn1*), *collagen, type I-α1* (*ColI-α1*), and *transforming growth factor-β1* (*Tgf-β1*). Administration of EVs also rescued the renal function as evidenced by the reduction in the blood urea nitrogen (BUN) and serum creatinine (SCr) levels [108]. Although the study conclusively demonstrated the salutary effect of EVs in a mouse model of AKI, the molecular mechanism involved in the process has not been elucidated.

Multiple sclerosis (MS) is characterized by inflammation, demyelination, gliosis, and axonal loss. Clark et al. (2019) used an induced experimental autoimmune encephalomyelitis (EAE) murine model of MS to assess the immunomodulatory properties of hCV-MSC (isolated from second semester placenta, grown in the presence of bFGF and EGF, early passage cells were used) and their EVs (high dose, 1×10^{10} EVs). They found that the treated mice (hCV-MSC or high dose EVs) displayed improved motor function outcomes, reduced DNA damage in oligodendroglia populations, and increased myelination within the spinal cord, as compared to animals treated with saline. The *in vitro* studies done with oligodendrocyte precursor cells (OPC) demonstrated that hCV-MSC-EVs promoted myelin regeneration by inducing endogenous oligodendrocyte precursor cells to differentiate into mature myelinating oligodendrocytes [15]. Whether the hCV-MSC isolated from full-term placentae and their EVs exert similar effects needs to be examined. Likewsie, whether the observed effects were due to priming of hCV-MSC with bFGF and EGF used in the culture medium needs to be looked into. However, these data suggest that hCV-MSC-EVs could be a viable option for the treatment of neurodegenerative diseases.

Presently, spinal cord injury (SCI) does not have any effective treatment. Taking a cue from the work done with hBMSC-derived exosomes on SCI, Zhang et al. (2020) examined whether hPMSC-derived (region of the placenta not specified) exosomes could have similar effects. They examined the effect of hPMSC-EVs on angiogenesis, both, *in vitro* and *in vivo*, and also on the sensory and locomotor functions of mice suffering from SCI. They found that the EVs promoted tube formation and migration of HUVECs *in vitro* [110]. Intrathecal injection of EVs in SCI mice resulted in a significant increase in vessel number, vessel volume, and vessel connectivity in their spinal cord.

Decreased invasion of trophoblasts interferes with arterial remodeling and affects the vascular structure formation in early pregnancy, leading to reactive oxygen species formation and the inflammatory cytokine release. Recently, Yang et al. (2019) used exosomes isolated from hAF-MSC to identify the role of miRNAs present in human trophoblast cells and in a mouse model of preterm birth (PB). Using LPS-induced trophoblast cells, they showed that the miRNA from hAF-MSC-exosomes have anti-inflammatory properties and based on these data, they suggest that these exosomes can be used to treat various inflammatory conditions, including PB. They also transplanted hAF-MSC in a PB mouse model and found that infusion of hAF-MSC reduced the LPS-induced gene expression of inflammatory cytokines such as TNFα, IL1β, and IL6 [111]. However, this study does not provide any data to show that treatment with hAF-MSC or their exosomes prevents PB.

Collectively, these studies demonstrate salutary effects of hPMSC-EVs on a range of different difficult-to-treat conditions like DMD, Parkinson's disease, acute kidney injury, MS, etc., and therefore, it is imperative that these leads should be taken forward to clinical trials. However, it is necessary to define the type of EVs used to generate pre-clinical data and to identify the active principle involved in the effect seen. It would then become possible to accurately map the macromolecular content of the EVs so that various batches can be standardised. It may also be noted that the growth factors used in the culture media could act as priming agents and could influence the EV content. Therefore, the culture conditions of the parental hPMSC need to be taken into account when ascribing any property to the EVs collected from any particular type of MSCs. Large-scale production of EVs having a consistent and desirable macromolecular profile, coupled with their effective cryopreservation, could pave the way to successful acellular therapy.

6. Future perspective

The placenta as a source of biologic has attracted the attention of researchers working in the area of regenerative medicine because of its easy availability, non-invasive methods of collection, fewer ethical issues, relatively large size, and, most importantly, the immense regenerative properties of the hPMSC and their EVs demonstrated in various studies. However, several issues still need to be addressed.

The placenta is a complex tissue composed of both maternal and fetal tissues, and the MSCs derived from these various parts have been shown to have somewhat different properties. Therefore, in every study, the region of the placenta used to derive hPMSC needs to be specified. Yet another aspect to be critically considered is the health of the mother! Since the condition of the mother gets reflected in the functionality of the hPMSC, it is of utmost importance that the mothers' health parameters such as age, preeclampsia, gestational diabetes, etc. are carefully noted down and the placentae are collected from a healthy mother giving birth to a healthy baby. However, the placentae from mothers suffering from diabetes, preeclampsia, etc.

Table 4
Clinical trials using human placenta-derived mesenchymal stem cells.

Sr No	Intervention /Condition/experimental system	Outcome	Dosage & route of administration	Reference
1	Effect of placenta-derived decidua stromal cells (hDSC) on acute graft-versus-host disease (GvHD) post allogeneic hematopoietic stem cell transplantation (HSCT)	38 patients with severe acute gastrointestinal GvHD were treated with hDSC. Among the steroid-refractory patients, overall response at 4 weeks after the DSCs intervention was 100 % and 7 of 11 patients had a complete response after 4 weeks. One-year survival was significantly better for patients treated with hDSC, as compared to those treated with bone marrow mesenchymal stromal cells (hBMSC).Severe adverse events reported included invasive fungal infections, relapse, pneumonia and other complications seen among HSCT patients.A pilot study done in 6 pediatric patients suffering from steroid refractory GvHD showed a complete remission in 4 patients at 6 months, while a partial response was seen in 2 patients. Five-year survival was 67 %. None of the children dies of GvHD.	hDSC 1×10^6 cells/kg in saline containing 5% human serum albumin were infused intravenously using a central venous line	90,91Clinical Trial No. NCT02172924, NCT02175303
2	Study the safety and efficacy of placental mesenchymal stem cells (hPMSC) in knee osteoarthritis (OA) healing	20 patients with knee OA were treated with hPMSC in a double blind, placebo-controlled study.Significant improvement in knee ROM and reduction in pain was seen between 2- and 24-weeks. Quality of life, activity of daily living, sport/recreational activity and decreased OA symptoms in the hPMSC-injected group was seen.Patients treated with hPMSC showed an increase in cartilage thickness compared to control No ectopic mass formation or any other adverse side effects were seen in subjects post 24 weeks.	hDSC 1×10^6 cells/kg in saline containing 5% human serum albumin were infused intravenously using a central venous line	92IRCT registration number: IRCT2015101823298N2
3	Study to evaluate the safety and efficacy of placenta- derived MSCs (hPMSC) for the treatment of critical limb ischemia (CLI) in diabetics	None of the four diabetic patients in the pilot study showed any serious adverse events such as infection at the site of injection or allergic reactions after hPMSCs treatment. Haematological, liver and renal biochemical functions, tumor markers, and electrocardiogram tests did not reveal any abnormality during the 24-weeks follow-up period. Resting pain scores, limb coldness scores decreased from base- line to 24 weeks after hPMSC therapy while pain-free walking distance significantly increased. Resting ankle brachial index and collateral vessel formation also increased in the subjects. Serum levels of TNF-α showed a significant decrease post-treatment.	1×10^6 hPMSC /kg were injected intramuscularly into the bilateral lower limbs (0.5–1.0 mL hPMSC per site, distance between two injections was kept as 3 cm × 3 cm. Total of 3 doses at an interval of 4 weeks.	93Chinese Clinical Trial Registry no. ChiCTR-ONC-16008732)

could be used to study the pathophysiology of the respective disorders. Another issue to be considered is whether the hPMSC derived from placenta obtained from a full-term delivery has better regenerative properties or those derived from the early gestation placentae, typically obtained in elective abortions: the latter would invoke more legal, ethical, and religious concerns.

In addition to these issues, the biochemical status of the cultured hPMSC needs to be carefully examined and the signalling gamut of various batches of hPMSC needs to be recorded, as the signalling mechanisms prevailing in the cells determine the macromolecular composition of their EVs [99,41]. One also has to determine whether priming of the cultured hPMSC is needed to accrue better therapeutic effects on the target tissues.

In addition to these hPMSC-related aspects, the specific issues with the use of their EVs also need to be considered. First and foremost is to define which type of EVs were used in the study; micro-vesicles or exosomes. Secondly, the macromolecular composition of these vesicles needs to be mapped, and active therapeutic principle(s) needs to be identified. The desired levels of the active entity in the vesicles to be clinically applied should be defined and should be used as a QA/QC parameter for each batch of the EVs collected. Optimization of the purification process to achieve large-scale preparation of endocytosis competent EVs [112] and their effective cryopreservation [113] also form some of the crucial aspects of this cell-free therapy.

The current COVID-19 pandemic has stimulated research on the use of cell therapy treating patients suffering from various side effects of this unusual viral infection, and clinical trials are being quickly undertaken by reducing the period required for various ethical and safety approvals [114–117].

This pandemic has given a clear signal that the world needs to be prepared on all fronts for such emergencies: cell therapy trials also need to be fast-tracked! There is an urgent need to prepare an international guideline covering the parameters mentioned above, but not limited to, so that it might be possible to have an inter-laboratory exchange of data. This could help the field move faster, rather than being stuck in the early phases of pre-clinical studies.

Author Contribution statement

PP and VK researched the literature and wrote the manuscript. VK conceived the contents, wrote, edited, and approved the final version. Both authors contributed to the article and approved the submitted version.

Declaration of Competing Interest

The authors report no declarations of interest.

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