



Placental mesenchymal stromal cells as an alternative tool for therapeutic angiogenesis

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Abstract

Dysregulation of angiogenesis is a phenomenon observed in several disorders such as diabetic foot, critical limb ischemia and myocardial infarction. Mesenchymal stromal cells (MSCs) possess angiogenic potential and have recently emerged as a powerful tool for cell therapy to promote angiogenesis. Although bone marrow-derived MSCs are the primary cell of choice, obtaining them has become a challenge. The placenta has become a popular alternative as it is a highly vascular organ, easily available and ethically more favorable with a rich supply of MSCs. Comparatively, placenta-derived MSCs (PMSCs) are clinically promising due to their proliferative, migratory, clonogenic and immunomodulatory properties. PMSCs release a plethora of cytokines and chemokines key to angiogenic signaling and facilitate the possibility of delivering PMSC-derived exosomes as a targeted therapy to promote angiogenesis. However, there still remains the challenge of heterogeneity in the isolated populations, questions on the maternal or fetal origin of these cells and the diversity in previously reported isolation and culture conditions. Nonetheless, the growing rate of clinical trials using PMSCs clearly indicates a shift in favor of PMSCs. The overall aim of the review is to highlight the importance of this rather poorly understood cell type and emphasize the need for further investigations into their angiogenic potential as an alternative source for therapeutic angiogenesis.

Keywords Placental mesenchymal stem cells · Stem cells · Wound healing · Exosomes · Secretome · Conditioned media · Immunomodulation · Differentiation potential · Ischemia · Hypoxia · Vasculature · Pregnancy · Cell based therapy · Regenerative medicine · Clinical trials

Clinical demand for angiogenesis

Angiogenesis is the process of formation of new blood vessels from pre-existing vasculature and is observed throughout the lifetime of an individual. Beginning with the earlier studies of Judah Folkman, a great interest developed over the years in understanding the regulation of angiogenesis as a key therapeutic tool [1–3]. While inhibition of angiogenesis is therapeutic for cancer, rheumatoid arthritis, etc., the enhancement of angiogenesis is a prerequisite, in a long list of disorders characterized by the lack of proper vascular development as seen in heart and brain ischemia, myocardial infarction, limb ischemia, diabetic wounds, pre-eclampsia and so on. Furthermore, age-related decline in vascular endothelial growth factor (VEGF), a key regulator of angiogenic signaling, could also lead to several complications [4]. Therefore, unlike what was thought of earlier, it has become very evident that angiogenesis and angiogenic factors play an important role in several clinical disorders.

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Around two decades ago, a new term was coined called ‘Therapeutic Angiogenesis’, defined as the means to stimulate angiogenesis for clinically relevant outcomes. This concept was further developed as a result of several experimental studies providing compelling evidence that certain factors like specific growth factors, oxygen regulation, pharmaceutical agents, bioactive matrices and other tissue-engineered products could be used to enhance the angiogenic potential at the sites of injury [5, 6]. Currently, researchers are turning toward cell-based therapies and specifically the use of mesenchymal stromal cells (MSCs) as an alternative tool for ‘natural systems of tissue repair’ [7]. Among several sources of MSCs, the placenta is now being considered a possible option. This review focuses on understanding the relevance of using placenta-derived MSCs (PMSCs) as a possible alternative cell source for therapeutic angiogenesis, the characteristics that set them apart, the means of enhancing their angiogenic potential and the main challenges that surround the use of these cells.

Placental mesenchymal stromal cells

It was A. J. Friedenstein and his colleagues, back in the early 1970s, who were the first to report on the significance of MSCs, which are multipotent, self-renewing and undifferentiated cells [8]. Although bone marrow-derived MSCs (BMMSCs) are considered a gold standard among other MSCs, the difficulty in obtaining BMMSCs has forced scientists to a hunt for new and versatile sources. MSCs from several sources have angiogenic potential [9, 10]. Indeed, MSCs from several sources have attracted a lot of attention in the clinical setting as a sought after cell-based therapy for acute diseases [11]. Among them, the placenta is gaining a lot of attention as an alternative source of MSCs, owing to its abundance and ease of availability. The placenta is

a transient feto-maternal organ, disposed off after delivery and involves no invasive procedures, making it ethically a more favorable source. A large recovery of PMSCs from a small tissue chunk has been also reported [12, 13]. It is also noteworthy that unlike most human tissue, the placenta is known to have lower levels of DNA methylation, making it more compatible with the human genome [14]. Moreover, as the placenta is a highly vascular organ, it is logical to assume that MSCs isolated from them should possess more angiogenic properties [15, 16]. This assumption is further supported by studies that show a direct contribution of the mesenchymal cells in the placenta to the formation of the early progenitors of hemangiogenic cells, rather than the fetal blood cells. Hemangiogenic cells form the first vessels in the placenta, providing further evidence of the putative role of PMSCs in angiogenesis [17].

Sources of PMSCs

The placenta is a disk-like structure with a diameter of 16–20 cm, thickness of 2–3 cm and weight an average of 500 g [12]. The placenta as a materno-fetal organ consists of both fetal and maternal parts (Fig. 1). The fetal parts include the chorionic plate (the fetal side of the placenta covered by the amnion), the amnion (covering the chorionic plate) and the umbilical cord (consists of two arteries and vein immersed in the Wharton’s jelly surrounded by the amniotic epithelium). The fetal membranes, the amniotic membrane (transparent, smooth, avascular and single-layered membrane composed of epithelium and mesenchyme) and chorionic membranes (composed of fibroblasts and trophoblast cells) further extend from the edges of the placenta and surround the fetus embedded in the amniotic fluid. The chorionic villi originate from the chorionic plate into the cotyledons. On the other hand, the maternal part is primarily formed of the decidua basalis [18]. The basal plate region of

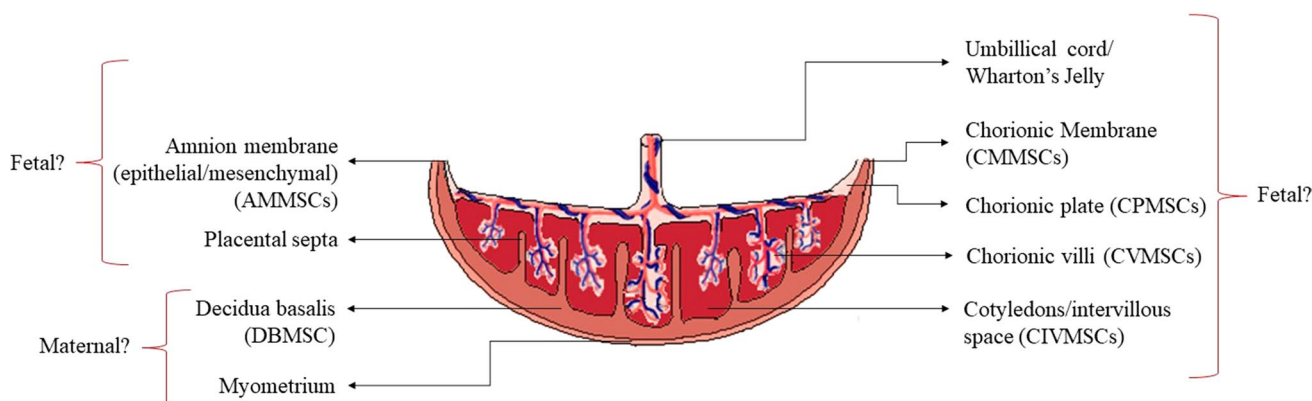


Fig. 1 Sources of placental mesenchymal stromal cells. The anatomy of the human term placenta, depicting major regions from which placental mesenchymal stromal cells (MSCs) have been derived. The amniotic fluid and the placental membranes have not been depicted

the decidua basalis protrudes into the chorionic villi region forming approximately 15–20 separations called the placental septa. The placental septa in turn partitions the parenchyma in the chorionic villi region into the cotyledons [12].

PMSCs have been used as a general term in referring to cells isolated from different sites of the placenta. Cells have been isolated from the decidua basalis (DBMSCs) [19], the chorionic membrane (CMMSCs) [20], the chorionic villi (CVMSCs) [21], chorionic cotyledons or intervillous space (CIVMSCs) [22, 23], chorionic plate (CPMSCs) [24], the chorionic trophoblastic cells [25], the amniotic membrane (AMMSCs) [26] and the amniotic fluid (AFMSCs) [27]. Since all these cells are from direct parts or extensions of the placenta, researchers sometimes refer to them generally as PMSCs, making it difficult to identify which specific cells have been used. Moreover, we only have a limited understanding of PMSCs from different sites of the placenta and only a few comparative studies have been performed to date [28]. We have tried to include all variants of direct isolations from the placenta, referring to specific types wherever available.

The PMSCs are often isolated via explant or enzymatic methods. In short, for the explant method, tissue from the respective source is dissected and washed with ice-cold phosphate-buffered saline to remove excess blood. The tissue chunks are minced and allowed to settle on culture dishes and the plates are then incubated with mesenchymal stem cell propagation media, in a humidified chamber with 5% CO₂ at 37 °C [29]. For the enzymatic method, the tissue was similarly minced and enzymatically treated with trypsin, while other protocols prefer an overnight collagenase treatment. After this, the isolated cells are seeded onto culture plates and maintained in the same way as that of the explant culture [30, 31].

Why PMSCs? A comparative note

MSCs isolated from different sources have specific differentiation potentials. We have presented a comparative understanding of different MSCs to PMSCs, based on properties that regulate angiogenesis.

Anatomical location MSCs are known to reside in several tissues and it is well documented that the niche in which they reside is a key regulator of the properties of that particular kind of stem cell [32]. Being a highly vascular structure, the placenta is exposed to enhanced levels of angiogenic signals that need to be maintained throughout the gestational period in response to fetal demands [33]. The placenta thus provides a suitable niche for pro-angiogenic signals. Moreover, researchers have shown that PMSCs reside primarily in the vascular niche of the placenta [15]. The highly vascular niche, suggests a higher potency of PMSCs for angiogenesis.

The placenta is also a rich source of other cells such as hematopoietic stem cells [34].

Source of fetal stem cells While ethical concerns restrict the use of embryonic stem cells, the use of fetal stem cells has been considered as a possible option. Fetal stem cells have been proven to be more primitive and more potent than their adult counterparts, making the use of fetal cells more effective in clinical applications [35]. The placenta is fittingly a rich source of fetal stem cells, an intermediate state between the adult stem cells and the embryonic stem cells.

Proliferative ability and migratory ability PMSCs have been shown to be more robust and proliferative and have more long-term growth ability when compared to their BMMSCs counterparts [30, 36, 37]. Several of their regulatory pathways have also been explored [38]. For instance, BMMSCs have a greater migratory capacity when compared to PMSCs [39], indicating that the BMMSCs can cross the endothelial blood vessel barrier more efficiently than the PMSCs [21]. Nonetheless, co-cultures of PMSCs, but not BMMSCs, with endothelial cells (ECs) induce migration of the ECs [40].

Differentiation potential Among the differentiation lineages, AMMSCs and CPMSCs have higher osteogenic and chondrogenic potential, but not adipogenic when compared to BMMSCs [20]. On the other hand, CPMSCs were more adipogenic, but not osteogenic when compared to the Wharton's jelly-derived MSCs (WJMSCs) counterparts [24]. Whether these differences in their basic differentiation potential would influence the angiogenic differentiation of these cells has not yet been established.

Immunomodulatory properties of MSCs One of the most striking features of MSCs is their immunomodulatory ability: a unique way of escaping and modulating the immune system [41]. Adult MSCs may express intermediate levels of HLA major histocompatibility complex (MHC) class I proteins, but lack MHC class II proteins on their cell surface [41]. Like other MSCs, PMSCs also have limited expression of HLA-A, B, C, making them less immunogenic and thus suitable for transplantation. A comparative study concluded that PMSCs have an advantage over several other MSCs including the BMMSCs [42], due in part to their immunomodulatory properties [43]. Stimulation of BMMSCs, umbilical cord MSCs (UCMSCs), and adipose-derived MSCs (ADMSCs) with interferon- γ (IFN- γ) resulted in an upregulated expression of MHC class II, while even a 3 day stimulation only showed a minimal expression in PMSCs [19]. This further supports earlier reports that PMSCs are more immunomodulatory compared to UCMSCs [42]. PMSCs regulate macrophage differentiation from an inflammatory M1 state to an anti-inflammatory M2 stage [44, 45]. The PMSCs also express (HLA)-G, which mediates inhibition of T cell proliferation [46]. A comparative study between conditioned media of cord blood-derived MSCs

(CBMSCs) and PMSCs on mononuclear cells and dendritic cells demonstrated a significantly pronounced reduction in T cell proliferation by PMSCs [42], due in part to transforming growth factor-beta and interleukin [47] or mediated by indoleamine-pyrrole 2,3-dioxygenase [48]. Although not much is known of the PMSC interaction with B cells, there are reports that state the prevention of early apoptosis of B cells. PMSCs also mediate immunosuppressive effects on NK cells [49]. Another key step during an inflammatory stimulation is the activation of the endothelium—involving the enhanced proliferation and upregulation of adhesion and inflammatory markers by the endothelium. DBMSCs, have a protective role under inflammatory conditions by reversing the effect of H₂O₂ on ECs [50]. Another group similarly demonstrated that T cell-mediated reprogramming of macrophages led to enhanced angiogenesis in mice with hind limb ischemia [44]. These studies are perhaps indicative of the fact that immunomodulation is one of the means by which PMSCs regulate angiogenesis [51].

Clonogenic ability of PMSCs The ability of a cell to form colonies may also be considered as a reflection of the ability of the cell to grow in a density-intensive fashion and is regarded as a mark of a genuine stromal cell [52]. A comparative study of CBMSCs demonstrated an increased formation of colonies by PMSCs when compared to CBMSCs isolated from the same tissue. This may also be reflective of the higher expansion ability of PMSCs [42].

Collectively, although the PMSCs are not highly migratory in nature, their anatomical location, proliferative ability, clonogenic ability and their high degree of immunomodulatory properties, make them ideal candidates for cell-based therapy. Moreover, a comparative study on the cytokine levels indicated a compatible level of VEGF and basic fibroblast growth factor (bFGF) secretion in PMSCs and human umbilical vein endothelial cells (HUVECs) when compared to UCMSCs and even BMMSCs [53] and a higher endothelial differentiation ability of CVMSCs than that of BMMSCs [54]. However, there is a need to optimize the ideal isolation and culture conditions such that bona fide fetal MSCs are grown.

Angiogenic ability of placental mesenchymal stromal cells

MSCs are now an accepted tool for therapy in case of dysfunctional angiogenesis [55] and PMSCs are gaining a lot of interest as a suitable cell source [56, 57]. The placenta is a vascular tissue, and it is therefore probable that MSCs isolated from the placenta may prove to be more potent for therapeutic angiogenesis. Apart from its general properties, their secretion of angiogenic factors, migration and secretion of matrix metalloproteinases to mediate the migration and

capillary tube formation further suggest their suitability for therapeutic angiogenesis (Fig. 2).

PMSC cell-mediated angiogenesis

PMSCs as a cell-based system for therapeutic angiogenesis is promising [58–60] (Table 1).

Differentiation into vascular-like cells

Capillary tube formation is a method employed to determine the cell's ability to differentiate into tube-like structures or stimulate capillary tube formation in ECs, and is used to measure in vitro angiogenic potential of cells. Cells are often observed for tube formation on Matrigel (growth factor reduced) surfaces and further quantified for the number of branch points, nodes, junctions, etc. [61]. Several studies have shown that PMSCs are capable of forming tubes in vitro (Fig. 3) on Matrigel without any additional cellular support. However, expression of markers such as von Willebrand factor (vWF) [62] was not seen either in CVMSCs or AMMSCs, suggesting a possible resistance toward differentiation into mature endothelial cells. However, a few studies indicate that post-induction, PMSCs were shown to acquire endothelial morphology and expressed vWF [53]. Interestingly, co-culturing of ECs with CVMSCs resulted in enhanced capillary tube formation, where PMSCs were seen to incorporate into the ECs in vitro [16]. CVMSCs also cross talk with ECs that expressed platelet-derived growth factor receptor-β (PDGF-BB receptor-β) via PDGF-BB signaling, which resulted in signal transducer and activator of transcription 3 (STAT3) activation of PMSCs and thus advanced formation of the vascular network [60] (Fig. 4). Moreover, following transplantation of labeled PMSCs, the cells were able to associate and incorporate into the mural layer of mouse placental vasculature and also expressed smooth muscle cell markers. Similarly, transplantation of green fluorescent protein (GFP)-labeled PMSCs revealed dual staining of GFP with αSMA and vWF in frozen sections of the muscle [53]. This signifies a synergistic role of PMSCs along with ECs and the possibility of direct differentiation of PMSCs into vascular cells that further highlights the engraftment potential of these cells.

In addition, PMSCs were seen to activate pericytes, which are contractile cells that wrap around blood vessels to provide contractility. A certain percentage of PMSCs exhibit pericyte-like phenotype, suggesting that PMSCs can give rise to progenitor cells that are responsible for the maintenance and repair of vessels, thereby contributing to stabilizing capillary tubes [15]. Furthermore, PMSCs can also differentiate into smooth muscle cells which aid in remodeling and stabilizing the vasculature [16]. The in vitro differentiation ability of PMSCs was dependent on the matrix

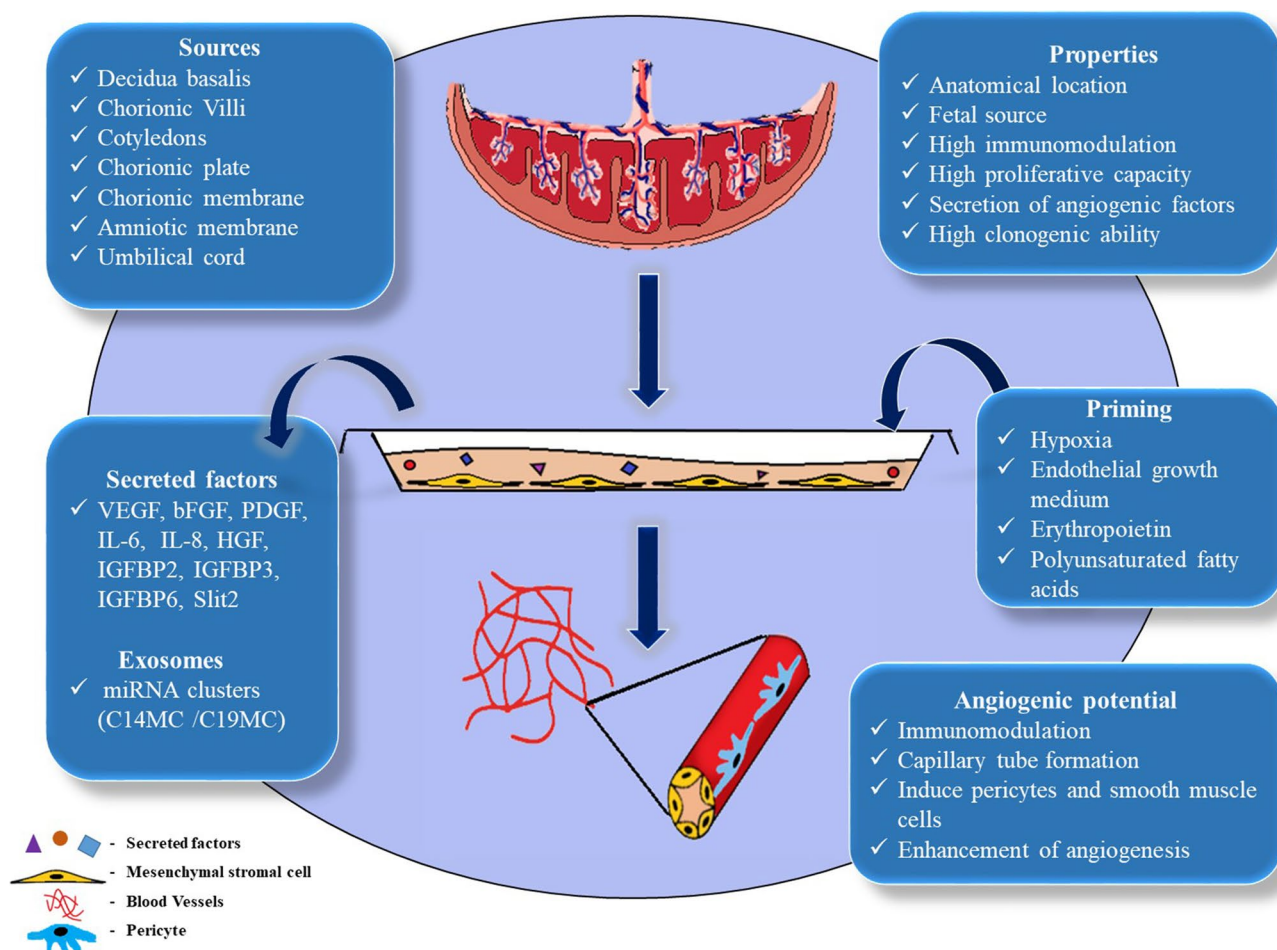


Fig. 2 Placental mesenchymal stromal cells and angiogenesis. Pictorial outline describing the different sources and properties of placenta-derived mesenchymal stromal cells (PMSCs). PMSCs can

be primed to enhance their angiogenic potential. The cells or their secretome can be applied to promote angiogenesis

on which it was coated with collagen I, collagen IV and laminin, showing improved differentiation into smooth muscle cells [16].

The question as to how exactly PMSCs modulate angiogenesis is still unclear—do PMSCs themselves differentiate into endothelial-like cells or is it more of an exocrine effect regulating the endogenous niche cells to mediate angiogenesis? Although there is need for more clarity on whether PMSCs themselves differentiate into mature endothelial cells, their paracrine influence on other cell types is certain.

PMSC secretome-mediated angiogenesis

Secretion of pro-angiogenic factors

MSCs are known to secrete a plethora of bioactive molecules that can enhance cell survival and are immunosuppressive, immunomodulatory and angiogenic [63]. PMSCs co-cultured with endothelial progenitor cells (EPCs) enhanced the

angiogenic potential of the EPCs via PDGF and NOTCH signaling [60]. Conditioned media (CM) collected from PMSC cultures have significant pro-angiogenic effects on EPCs and HUVECs [57]. Interestingly, a comparative study revealed MSCs derived from blood vessels (CVMSCs) were able to stimulate angiogenesis of ECs to a greater extent than MSCs from avascular sources (AMMSCs) [62], highlighting the importance of determining the components of the CM from different cell sources (Table 1).

Among the angiogenic factors, PMSCs express VEGF (302 ± 25.79 pg/mL) [60], bFGF (50 ± 30.72 pg/mL) [60], angiogenin, IL-6, IL-8, HGF, IGFBP2, IGFBP3 and IGFBP6 [57, 62]. The possible mechanisms of angiogenesis reported include activation of pAkt and p38MAPK/pSTAT3 that activate VEGF secretion [64]; PDGF-BB (vascular maturation and recruitment of smooth muscle cells and pericytes) [16] that enhance the angiogenic potential of endothelial cells by upregulating angiogenic markers such as Tie2 and Ang2 [57] and the role of stromal cell (PMSCs)-derived Slit2

Table 1 Placental Mesenchymal Stromal Cell mediated angiogenesis

	Source	Study	References
<i>Placental mesenchymal stromal cell</i>			
Laboratory work	Decidua-derived MSCs	MiR-136 inhibits angiogenesis targeting VEGF	[103]
	Placental cotyledon MSCs	Mediate placental angiogenesis via PDGF-BB and STAT3 pathway	[22]
In vivo studies	Blood vessel-derived PMSCs	Stimulate angiogenesis	[62]
	Intervillous MSCs	Supplement with PUFA-enhanced angiogenesis via VEGF in mice model	[91]
	Placenta-derived MSCs (not defined)	Direct participation of PMSCs via differentiation into vascular cells and cytokine release	[53, 60]
	Placenta-derived MSCs (not defined)	Enhance angiogenesis via T cell-dependent reprogramming of macrophage differentiation	[44]
	Placenta-derived PLX-PAD MSCs	Enhance angiogenesis in hind limb ischemia mouse model in a dose- and site-dependent manner	[56]
	Placenta-derived adherent cells (PDA-002)	Increased number of blood vessels, improved blood flow and vascular density in hind limb ischemia mice models	[97]
	PMSCs	Enhances angiogenesis after ischemic limb injury in mice model	[98]
	PMSCs from villous tissue	Incorporation of PMSCs into vessel walls in mouse placenta	[16]
Clinical trials	Placental cotyledon-derived MSCs	Angiogenesis in differentiated placental multipotent mesenchymal stromal cells is dependent on integrin $\alpha 5\beta 1$	[38]
	Intramuscular injections of placenta-derived adherent cells (PLX-PAD)	Phase 3: critical limb ischemia (CLI)	NCT03006770 https://clinicaltrials.gov
	Intramuscular Injections of placenta-derived adherent cells (PLX-PAD)	Phase 1: peripheral artery disease, peripheral vascular disease, critical limb ischemia	NCT00919958 NCT00951210 https://clinicaltrials.gov
	Intramuscular injection of human placenta-derived cells (Pda-002)	Phase 2: peripheral arterial disease, diabetic foot	NCT02264288 https://clinicaltrials.gov
<i>Placental mesenchymal stromal cell-conditioned media</i>			
Laboratory work	Placental cotyledon-derived MSCs	Modulate placenta angiogenesis through Slit-Robo signaling	[22]
	Placenta-derived mesenchymal stem cells	Conditioned medium of H9c2 triggers VEGF dependent angiogenesis by activation of p38/pSTAT3 pathways	[64]
In vivo studies	Chorionic plate and chorionic villi MSCs	Incorporation of placental MSC-derived exosomes into endothelial cells enhancing its tube formation and migration in murine auricle ischemic injury model	[57]
	Chorionic villi MSCs	VCAM1 + MSCs display enhanced angiogenic paracrine activity in hind limb ischemia mice models	[107]
Clinical trials	Nil		

expressed by PMSCs in enhancing endothelial tube formation via Roundabout (Robo) receptors present on endothelial cells [22] (Fig. 4).

CM of PMSCs have other properties that improve transplantation. Cell viability is enhanced coupled with reduced oxidative stress, when CM of PMSCs are deployed along with the cryopreservation mix. The

priming of cells with the PMSC-derived CM improves the transplantation ability of cells when compared to CBMSC-derived CM [65]. There are several studies supporting the concept that tissue repair is likely to be an atrophic effect mediated by the secretion of factors, rather than the differentiation of the cells themselves [66, 67].

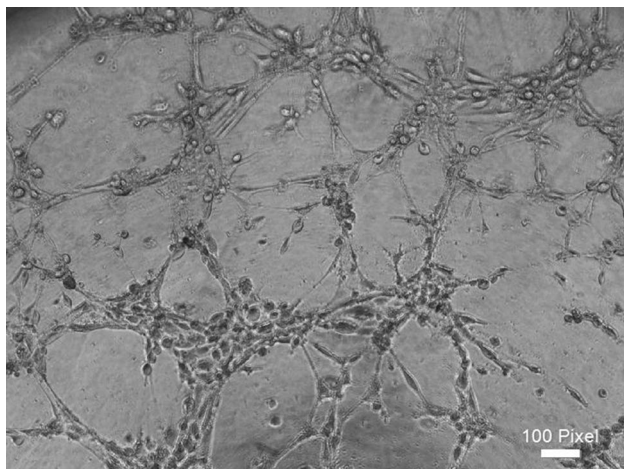


Fig. 3 In vitro tube formation of placental mesenchymal stromal cells. PMSCs showing tube formation potential post-24 h culture on Matrigel

Role of exosomes

Current research is now shifting gears and looking at more specific components of the CM, most notably the exosomes. These are extracellular vesicles, much smaller than the microvesicles (> 200 nm), ranging from 50 to 200 nm in diameter, processed through several endocytic steps before being released and are known to be involved in several functions [68, 69]. Exosomes contain proteins or RNA and can be prepared from CM of MSCs by ultracentrifugation [57]. PMSC-derived exosomes (PMSC-exos) are known to influence osteogenic and adipogenic differentiation by upregulating OCT4 and NANOG in dermal fibroblasts [70]. They enhance tube formation and migration of endothelial cells [57]. Incorporation of labeled exosomes into the endothelial cells demonstrated the involvement of exosomes in mediating these changes.

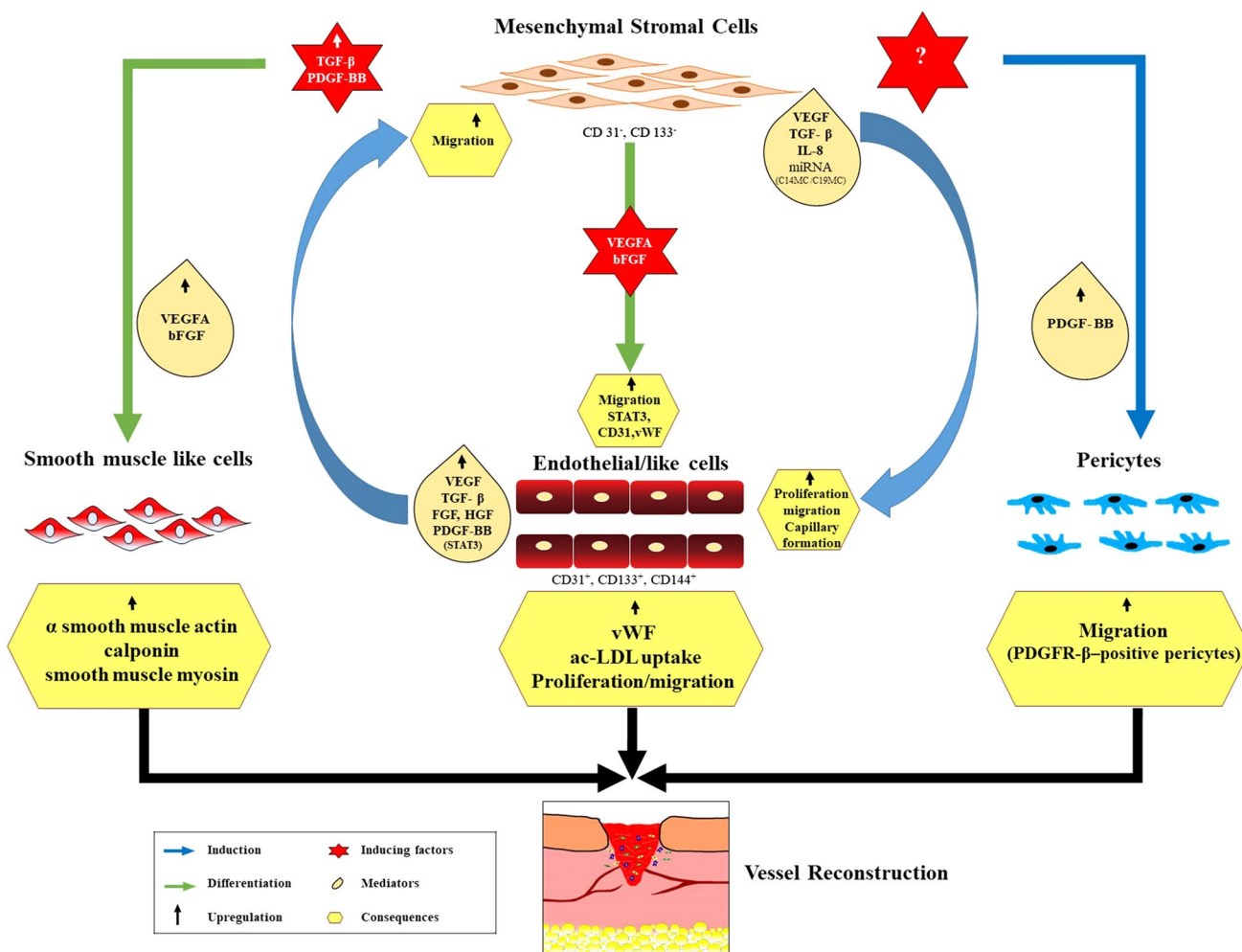


Fig. 4 Possible mechanism of placental mesenchymal stromal cells contributing to angiogenesis. Possible mechanism behind the role of PMSCs mediating angiogenesis via direct de-differentiation or

through its paracrine effects on effector cells such as the smooth muscle cells, endothelial cells and the pericytes in forming mature vessels

Possible exosomal mechanisms include transfer of miRNAs into the endothelial cells that subsequently promote angiogenesis [71] and may be responsible for up to 60% of the angiogenic potential of the CM [57]. miRNAs are 20–24 nt-long non-coding RNAs that mediate a sequence-specific degradation of mRNA [72]. Most of the placenta-specific miRNAs are derived from the chromosome 14 and chromosome 19 miRNA clusters (C14MC/C19MC) [73], which are the largest gene clusters of human miRNAs [74]. Several studies have implicated the role of miRNAs in angiogenesis, miR-17-92 cluster (tumor angiogenesis), miR-27b [75], bLet7-f [75], miR210 and mir-130a. However, specific miRNAs from PMSC-exos targeting endothelial cell function and angiogenesis are yet to be identified. There are, however, reports indicating the role of miRNA125a (adipose-derived exosome) in repressing the angiogenic inhibitor delta-like 4 (DLL4) in endothelial cells and thereby promoting angiogenesis [76]. Although several studies use CBMSCs [77], there are fewer studies reported with PMSCs and therefore a more detailed analysis needs to be done on the potential role of placenta-specific miRNAs. Moreover, as exosomes are not static but highly diverse [69], the relevance of the niche of cells and the protocols used are also being intensively investigated. However, further studies are needed to validate this, but if proven these studies may open up new avenues for the use of cell-free therapy [78].

There is a lot of attention on cell-free or secretome-based therapy with the prospect that the native endogenous cells could be triggered to respond to an injury [79]. However, in most degenerative diseases such as diabetes and among patients undergoing chemo/radiotherapy, the MSC niche is compromised [80]. There have also been reports of age-related decline in the quantity and quality of endogenous MSCs [81]. Under such circumstances, the use of cells over that of CM may become the preferred choice of therapy, thereby making the selection of cell or cell-free based therapy a more case-dependent choice.

Induction of PMSCs to enhance angiogenesis

Although several outcomes under laboratory conditions are promising, actual pre-clinical experimentation has not been as successful. Angiogenesis is a complex process and requires the interplay of multiple factors [82]. Hence, researchers are trying to understand how the angiogenic potential of PMSCs can be made more effective. There is growing evidence that the differentiation of MSCs can be altered by varying their environment [83]. Scientists are now looking at priming cells with factors or physical environment suitable for enhancing their angiogenic potential to ensure success in cell therapy [84].

As placental development takes place under hypoxic conditions (rather normal conditions for placenta cells), one area

of focus is in considering the culture of cells under more physiologically relevant oxygen conditions [34]. The placenta has been documented to have a PO₂ of 20 mmHg in the first trimester [85]. Studies now indicate that hypoxic priming of PMSCs may enhance their angiogenic potential [23]. In this context, Xu et al. demonstrated how hypoxia mediated phosphorylation of JAK/STAT3 signaling-promoted angiogenesis in trophoblast cells [86]. Conversely, hypoxia decreased angiogenesis via Notch 1 signaling [87]. Another surprising study revealed that in differentiated syncytiotrophoblast, HIF2 α downregulated placental growth factor (PlGF) secretion [88], which is a key molecule in angiogenesis. It is clear from the available literature that the oxygen concentration is indeed relevant not only for in vitro culture of cells, but also in regulating how cells trigger angiogenesis in vivo. Nonetheless, more validation is required to establish this process in full. Several molecular interventions, such as compounds to increase the expression of CXCR4 [stromal cell-derived factor-(SDF-1) receptor], to enhance the migratory ability of MSCs [80], priming with endothelial growth medium [89], erythropoietin [90] and even pharmacological agents such as polyunsaturated fatty acids [91, 92] have been proposed. Alternatively, application of differentiated vasculogenic progeny to endothelial-like cells from PMSCs contributed to enhancing blood vessel formation in an integrin ($\alpha 5\beta 1$)-dependent manner [38].

It's therefore clear that PMSCs have an ability to form tubes in vitro and increased capillary formation in vivo. They can also induce other niche cells such as the ECs, pericytes and smooth muscle cells to contribute to angiogenesis via paracrine factors. The more recent studies using exosomes further confirm that both cellular and secretome-based applications are possible from PMSCs.

Applications

Pregnancy-related disorders

Severe dysfunctional vasculature is seen in many pregnancy-related disorders such as pre-eclampsia and gestational diabetes [93]. As pregnancy disorders are often related to trophoblastic abnormalities and since PMSCs are present in the placenta through term, there is increasing interest in considering PMSCs isolated from such placentae as tools to study the respective disorder [94]. Evidence of incorporation of transplanted PMSCs (human) in the vessel walls of mouse placenta further confirms the involvement of PMSCs along with endothelial cells during placentation [16]. Few relevant examples include studies that demonstrate that MSCs from the placenta of gestationally diabetic mothers are hampered in their glucose uptake and display premature aging and

altered angiogenesis, while MSCs from obese mothers have greater adipogenesis [95, 96].

Angiogenic potential

Several *in vivo* studies show promising results of usage of PMSCs in mice models, particularly in limb ischemia models. PMSCs possibly by direct differentiation and incorporation [16, 60] or via cytokine release [91] mediated an increase in the number of blood vessels and blood flow, enhanced vessel density and perfusion at the ischemic site [97, 98] in a very dose- and site-specific manner [56].

Anti-angiogenic potential

Conversely, co-culturing of CVMSCs along with breast cancer cells resulted in a significant reduction in proliferation, migration and tube formation ability of HUVEC cells [99]. Exosomes of MSCs suppress angiogenesis via downregulation of VEGFA [100], while PMSCs suppress peritoneal tumorigenesis [101]. AMMSCs, when injected into retinopathy mouse models, were able to suppress proliferation and prevent neovascularization via upregulation of TGF- β 1 [102]. This can partly be explained by the upregulation of miRNAs such as miR-136 [103] under pathology conditions that mediate inhibition of capillary formation. Therefore, it is important to note that PMSCs modulate angiogenesis in a very contextual manner.

Clinical trials

The first clinical trial to use culture-expanded MSCs was performed at the Ireland Cancer Center of University Hospitals of Cleveland [104] using bone marrow-derived MSCs in a phase I trial. Several studies have since been completed and the results are promising (<http://www.clinicaltrials.gov/>). Studies on the use of PMSCs for other complications such as pulmonary fibrosis (NCT01385644) and Peyronie's disease (NCT02395029) have been completed. The first of its kind company to harvest PMSCs as a therapeutic product is the Pluristem Therapeutics Inc., Israel, which validated the possibility of an 'off-the-shelf supply of therapeutic cells' isolated from the placenta. Their work initially suggested that intramuscular administration of PMSCs in a hind limb ischemia BALB/c mouse model resulted in reduced endothelial damage and enhanced limb function [105]. Further clinical trials using PMSCs, administered via intramuscular injections, for critical limb ischemia and peripheral vascular disease range from phase I to phase III and the results seem encouraging (Table 1).

Other applications include the role of PMSCs as feeder layer for the culture of hematopoietic stem cells—HSCs [106]. The results from the use of PMSCs have been so

promising that researchers are now even suggesting the establishment of placental cell biobanks [34].

Challenges

There is a lot of heterogeneity in the techniques adopted for PMSC isolation and consequently a lot of variation in the results, hence the need for proper determination of isolation techniques and means of characterization of the isolated cells. In addition, CVMSCs that were VCAM-1(+) were more promising for *in vivo* administration in ischemic mice models [107], further emphasizing the identification of key cells within the pool of isolated PMSCs.

Are PMSCs fetal cells? According to the First International Workshop on Placenta Derived Stem Cells, it is mandated that isolated cells be verified as of fetal origin with less than 1% contamination with maternal cells [12]. Although it is assumed that cells isolated from pre-natal sources are ideally fetal, there is very little documentation available among published literature on whether cells isolated from the placenta are indeed fetal. An interesting study reported the presence of fetal cells isolated from the amniotic membrane and maternal cells from the chorionic membrane [20]. Such uncertainty over the origin of cells can lead to variations in published results [108]. Indeed, reports that maternal cells overpopulate a culture of cells isolated from fetal villi clearly suggests that the mere choice of site of isolation would not guarantee the origin of the cells [109]. Researchers are now pointing out that even the protocol used for isolation [110] or the media used in expansion [109] is relevant in obtaining and maintaining pure fetal cells. Another important factor that could add to the differences is the maternal age [111], although it is not clear how this would influence fetal cells! This necessitates the need to standardize isolation protocols to obtain fetal cells and definitely a need to validate the cell cultures prior to experimentation.

Conclusions

In summary, PMSCs are readily obtainable, ethically favored and can be isolated with ease and high cell yields. Their mesenchymal characteristics are similar to that of the gold standard BMMSCs and are highly immunomodulatory. The cells and their secretome can promote angiogenesis by modulating the niche cells (Fig. 2). Although a few clinical trials have shown promise, PMSCs are far less understood and explored compared to their BMMSC counterparts. Nevertheless, while further studies are required to better understand the potential of these cells, the existing literature clearly indicates the potential of PMSCs as an ideal cell source for therapeutic angiogenesis.

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Author contributions Suja A. Mathew: conceived, designed and wrote the paper; Charuta Naik: collected the data and prepared the image; Paul A. Cahill: critical editing and writing of the paper; Ramesh Bhonde: designed and critical editing of the paper.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest in their study.

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