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Amniotic membrane and amniotic cells: Potential therapeutic tools to combat tissue inflammation and fibrosis?

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ABSTRACT

In addition to the placenta, umbilical cord and amniotic fluid, the amniotic membrane is emerging as an immensely valuable and easily accessible source of stem and progenitor cells. This concise review will focus on the stem/progenitor cell properties of human amniotic epithelial and mesenchymal stromal cells and evaluate the effects exerted by these cells and the amniotic membrane on tissue inflammation and fibrosis.

1. Introduction

1.1. Human amniotic epithelial and mesenchymal stromal cells

The human amniotic membrane (AM) contains two distinct cell populations. Human amniotic epithelial cells (hAEC) are cuboidal to columnar cells that form a monolayer lining the membrane and are in direct contact with the amniotic fluid. hAEC arise from the embryonic epiblast and are amongst the first cells to differentiate from the conceptus [1,2]. In contrast, human amniotic mesenchymal stromal cells (hAMSC) are dispersed in an extra-cellular matrix largely composed of collagen and laminin, and are derived from the extraembryonic mesoderm [1]. Both cell types originate during the pre-gastrulation stages of embryogenesis before the delineation of the three primary germ layers [1,2]. Embryonal carcinoma cells that are formed prior to gastrulation have been shown to retain stem cell-like properties. Thus, the early origin of the AM cells was a major reason that led to investigations into the plasticity and stemness of these cells.

Efficient protocols have been established for hAEC and hAMSC isolation from term placenta and are generally based on the separation of the AM from the chorionic membrane and subsequent enzymatic digestion [2–5]. A typical term AM yields between $150–200 \times 10^6$ hAEC and $20–50 \times 10^6$ hAMSC [6]. In culture, hAMSC exhibit plastic adherence and fibroblast-like morphology, while hAEC display a typical cobblestone epithelial phenotype. Many of the surface and intracellular stem/progenitor markers expressed by AM cells are listed in Tables 1 and 2. However, there is considerable variation in the percentages of AM cells expressing these markers reported by different investigators. The levels and pattern of marker expression appear to depend on the isolation protocol used and vary with expansion [7]. Gestational age dependant changes in marker expression have also been found. Surface markers such as CD44, CD49e and CD13 were significantly lower in hAEC derived from third trimester compared to first trimester [8] and hAEC expressing the pluripotency associated Nanog, Sox-2, Tra-1-60 and Tra-1-80 genes were higher in second trimester AM compared to term [9]. Further, cells with pluripotency associated markers have been found to be randomly distributed in the epithelial layer of term delivered AM [10]. This heterogeneity in distribution and gestational age dependant changes is also likely to contribute to different sub-populations being analyzed [2,5,8,11–14] and impact on investigations into stem cell properties and possibly pregnancy related studies using AM cells.

Interestingly, hAEC and hAMSC also express a repertoire of lineage associated genes (Tables 1 and 2), suggesting that they could act as progenitors and differentiate into various cell types. Indeed, hAEC and hAMSC have the ability to differentiate *in vitro* into cells from each of the three germ layers (Tables 1 and 2). After stimulating cells in media supplemented with growth factors, hormones and/or other additives, differentiation was monitored by evaluating the

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Table 1
Phenotype of undifferentiated hAEC and hAEC induced to differentiate *in vitro*.

Undifferentiated hAEC			
Marker groups	Markers	Detection methods	References
Mesenchymal and hematopoietic markers	CD10+, CD13+, CD29+, CD44+, CD49e+, CD73+, CD90+, CD105+, CD117+, CD166+, STRO-1+, CD14−, CD34−, CD45−, CD49d−, HLA-ABC +, HLA-DQ+/−, HLA-DR−	FACS	[2,7,11–15]
Stem cell markers	POU5F1 (OCT-4)+, Sox-2+	RT-PCR, immunocytochemistry	[2,11,14,15]
	FGF-4+, Rex-1+, CFC1+, Nanog+, DPPA3+, PROM1+, PAX6+, FOXD3−, GDF3−, TERT−	RT-PCR	[2,11,14,15]
	SSEA-3+, SSEA-4+, Tra 1-60+, Tra 1-81+, SSEA-1-	FACS, immunocytochemistry	[2,7,13–15].
	GCTM2+	immunocytochemistry	[11,15]
Neural lineage associated markers	Nestin, GAD, MBP, NF-M, NSE, CNPase, PLP, DM-20	RT-PCR	[2,14]
	Nestin, MAP2, GFAP	immunocytochemistry	[15]
	Neurofilament proteins, MAP2, MAP2 kinase	Immunocytochemistry	[39]
Lung associated markers	Nkx2.1, mucin, occludin, aquaporin-5, caveolin-1	RT-PCR, FACS	[11]
Hepatic lineage associated markers	Albumin, α-FP	RT-PCR, western blot, immunocytochemistry	[40]
	Albumin, α-1AT, CK18, GS, CPS-1, PEPCK, CYP2D6, CYP3A4, α-FP, TTR, TAT, CYP2C9, HNF3-γ, C/EBP-α	RT-PCR	[41]
Cardiomyogenic lineage associated markers	GATA-4, Nkx 2.5, MLC-2A, MLC-2V, MYL-7, ANP, CACNA1C, KCND3	RT-PCR	[14,15]
Pancreatic lineage associated markers	PDX-1	RT-PCR	[14]
Others	CD31-, CD324+ (E-cadherin), ABCG2/BCRP+, vimentin+, PanCK+,	FACS, immunocytochemistry	[2,11]

Differentiated hAEC			
Lineages	Characterization of differentiation: markers/cell morphology/tissue specific functions	Detection methods	References
Adipogenic	PPARγ; LPL; cells enlarge three/four times and are multinucleated; cells contain lipid deposits	RT-PCR, Oil Red O stain	[7,15]
Osteogenic	OSC, OSN; cells enlarge two/three times ad are binucleated; cells contain calcium deposits	RT-PCR, Von Kossa stain, Alzarin Red stain	[7,15]
Chondrogenic	Expression of collagen I and II; synthesis of proteoglycans	immunohistochemistry, Toluidine Blue Stain	[7]
Myogenic	ATCA2; MyoD1, skeletal muscle myosin heavy chain; elongated cells, multinucleated cells	immunocytochemistry	[8,15]
Neural	Increased expression of nestin, GAD, MBP, NF-M, NSE; expression of GFAP, CNP; elongated cells with neuronal- or astrocyte-like morphology	RT-PCR, immunocytochemistry	[14]
	Few nestin+ and MAP-2+ cells with neuronal morphology; numerous GFA + cells with astrocyte-like morphology	immunocytochemistry	[15]
Lung	Production of SPs A–D, SP-D secretion; epithelial phenotype with lamellar body formation	electron microscopy, immunohistochemistry, ELISA	[11]
Hepatic	Albumin; α-1AT, HNF-4α; CYP1A activity	RT-PCR, immunohystochemistry, EROD assay	[14]
	Albumin, HGF; features of hepatocytes	immunocytochemistry, FACS, transmission electron microscopy	[15]
Cardiomyogenic	GATA-4, MYL-7, ANP, CACNA1C, KCND3, TTNT; features of relatively mature cardiomyocytes	RT-PCR, FACS, transmission electron microscopy	[15]
	Increased expression of GATA-4, Nkx 2.5, MLC-2A, MLC-2V; expression ofα-actinin	RT-PCR, immunocytochemistry	[14]
Pancreatic	Increased expression of Pdx-1; expression of Pax-6, Insulin, NKx 2.2, glucagon	RT-PCR, immunocytochemistry	[14]
	AMY2B, glucagon; features of exocrine acinar beta cells	RT-PCR, immunocitochemistry, FACS, transmission electron microscopy	[15]

morphological changes (e.g. changes into cells with neuron, hepatocyte and cardiomyocyte-like features), expression of various lineage-specific genes, as well as assessing acquired abilities to exert tissue-specific functions (Tables 1 and 2). However, the level of maturation achieved *in vitro* may be variable [2,15], and could be due to the inability to express genes present in the terminally differentiated cells, shortcomings in the induction media, extra-cellular matrices, oxygen tensions and culture conditions used.

Clonal colony formation is an important feature of adult tissue derived stem cells. There are conflicting reports on the clonogenicity of AM cells. hAEC and hAMSC were found to be clonogenic with hAMSC forming colonies that could be expanded for at least 15 passages [5,15], whereas others report the absence of clonal colony formation by hAEC and hAMSC [13]. Generally, at much higher seeding densities, hAEC and hAMSC can be kept in culture for 5-10 passages [5,12,13]. Interestingly, after a few passages hAEC change from the cuboidal epithelial shape into elongated stromal-like cells that express markers associated with mesenchymal and fibroblast cells and show reduced differentiation potential [12,13]. The reason/s for these changes remains uncertain, but may be due to senescence, epigenetic modifications and to the autocrine/paracrine effects of growth factors such as EGF and TGFβ that are known to induce an epithelial to mesenchymal transition. Although the phenotypic changes are not as marked compared to hAEC, the morphology of hAMSC and differentiation potential also declines with expansion [5,12].

There is some evidence that AM cells can differentiate into cardiomyocytes, neural, alveolar epithelium and pancreatic β-islet cells following xenotransplantation and secrete proteins produced by hepatocytes [11,16]. Further, trophic factors secreted by hAEC and hAMSC could exert angiogenic, growth promoting, anti-inflammatory and anti-fibrotic effects following transplantation [16]. Thus, with a view to potential therapeutic applications, researchers are also developing isolation protocols in accordance with current guidelines for clinical use [16,17]. Culture of hAEC in

Table 2
Phenotype of undifferentiated hAMSC and of hAMSC induced to differentiated *in vitro*.

Undifferentiated hAMSC			
Marker groups	Markers	Detection methods	References
Mesenchymal and hematopoietic markers	CD3-, CD13+, CD14-, CD29+, CD34-, CD44+, CD45-, CD49e+, CD54+, CD73+, CD90+, CD105+, CD117 (weak), CD166+, $CD27^{low}$+, STRO-1+, HLA-A-B-C+, HLA-DR−	FACS	[1,2,7,8,13,42]
Stem cell markers	SSEA-3+, SSEA-4+	FACS, immunocytochemistry	[1,13]
	POU5F1 (OCT-4)+, Rex-1+, BMP-4+	RT-PCR	[1,13]
Endothelial marker	CD31-, VEGF receptor 1 and 2: FLT-1+ and KDR+	FACS	[1]
Hepatic lineage associated markers	Albumin, CK18, α-FP, α1-AT, HNF4α	RT-PCR	[43]
Pancreatic lineage associated markers	PDX-1	RT-PCR	[44]
Cardiomyogenic lineage associated markers	GATA-4, MLC-2A, MLC-2V, MLC-2v, cTnI, and cTnT, α-subunits of the cardiac-specific L-type calcium channel, Kv4.3	RT-PCR	[45]
Neural lineage associated markers	Nestin and musashi1, Tuj1 and NF-M, GFAP	RT-PCR, immunocytochemistry	[46]
Others	CD349+, CD140b+, CD324 (E-cadherin)-, vimentin+	immunocytochemistry	[2]

Differentiated hAMSC			
Lineages	Characterization of differentiation: markers/cell morphology/tissue specific functions	Detection methods	References
Adipogenic	LPL; accumulation of lipid deposits	RT-PCR, Oil-red O stain	[5]
Chondrogenic	Collagen-II; cartilage-specific metachromasia	RT-PCR, Toluidine Blue stain	[5]
Osteogenic	OPN; induction of calcium deposition	RT-PCR, Alizarin red stain	[5]
Myogenic	myoD, myogenin,desmin	RT-PCR, immunocytochemistry	[42]
	myoD1, skeletal muscle myosin heavy chain; features of myotubes	immunocytochemistry	[8]
Hepatic	Increased expression of albumin, CK18, α-FP, α1-AT, HNF4α; storage of glycogen	RT-PCR, immunocytochemistry; PAS staining	[43]
Pancreatic	Increased expression of Pdx-1, Isl-1, Pax-4, Pax-6, expression of insulin, glucagon, somatostatin; appearance of islet-like cell clusters	RT-PCR, immunocytochemistry	[44]
Cardiomyogenic	GATA-4, MLC-2A, MLC-2V, cTnI, and cTnT, α-subunits of the cardiac-specific L-type calcium channel, Kv4.3, induction of Nkx2.5, ANP and cardiac-specific gene -myosin heavy chain; integrate in cardiac tissues in co-culture experiments	RT-PCR, immunocytochemistry	[45]
Angiogenic	Increased expression of FLT-1, KDR, ICAM-1, appearance of CD34 positive cells, expression of vWF; features of endothelial cells	FACS	[42]
Neurogenic	Increased expression of nestin, musashi1, Tuj1 and NF-M, GFAP	RT-PCR, immunocytochemistry	[46]

serum free medium appears to lead to the expression of hematopoietic and monocytic markers, high telomerase activity and long telomere lengths [17], whereas hAEC that are routinely cultured in fetal calf serum (FCS) supplemented media lack these markers and telomerase activity [12–14]. Comparisons of cells grown in FCS with serum free media or media containing acceptable alternatives for clinical use such as platelet lysate and human serum may be warranted, as for example, a high level of telomerase activity is linked to teratoma and tumor formation. Injection of primary or passaged hAEC and hAMSC that have been cultured in FCS into mice, rodents and swine has so far not led to tumour formation [14,15,18–20], but the fusion of amniotic cells with host cells to form dysplastic precursors cannot be ruled out. Further, under serum free conditions a selection of surface markers were differentially expressed by primary and passage 5 hAEC [17]. This reinforces the possibility that culture conditions may select different cell populations, thereby altering the phenotype of the naïve population. Thus, further investigation would be beneficial as results reported on cell replacement, inflammation and fibrosis following xenotransplantion have been reported using primary hAEC and hAMSC prepared using FCS.

1.2. Pre-clinical studies investigating amniotic cells

Lung and liver fibrosis, myocardial infarct and stroke are leading causes of mortality and together with their long term morbidity places major burdens on health care systems worldwide. In pre-clinical animal disease models, human amniotic cells were found to make a modest contribution toward replacing damaged alveolar epithelium, endothelium and heart muscle [11,21,22], whereas a more significant contribution is likely to be their anti-inflammatory and anti-scarring effects.

Pooled MSC from amniotic and chorionic membranes and hAMSC alone have been injected directly into infarcted rat hearts following arterial ligation. Treated rats showed increased capillary density, improved left ventricular function and fractional shortening and a reduction in fibrotic scar tissue [21,22].

hAEC and hAMSC have also been evaluated as a treatment for liver and lung fibrosis. Hereditary, pathogenic, environmental and lifestyle factors can induce inflammation in liver and lungs and lead to collagen deposition in response to wound healing. The repeated insults lead to apoptosis and necrosis of cells, immune cell infiltration, release of pro-inflammatory cytokines, activation of resident cells into collagen depositing myofibroblast cells, altered tissue architecture and compromised organ function. Administration of Bleomycin, is widely used to mimic the phases of lung inflammation and fibrosis observed in patients with generic pulmonary fibrosis and acute respiratory distress syndrome. A 1:1 mixture of hAEC and hAMSC/human chorionic MSC was administered intra-tracheally or intra-peritoneally into Bleomycin-treated, immunocompetent C57/Bl6 mice [19]. Irrespective of the route of administration, human DNA and cytokeratin-19 positive cells were localized over the two week test period in lungs of mice receiving xenotransplants. Importantly, treated mice showed reduced neutrophil infiltration and fibrosis area whereas macrophage and lymphocyte numbers did not show significant changes [19]. Another study using Bleomycin injured SCID mice tested hAEC [11]. The data showed that following intravenous delivery, some hAEC persisted in the lungs over the four week test period, reduced IL-1, IL-6, TNFα protein levels and collagen in lungs and augmented regeneration leading to improved lung architecture [11].

The toxin carbon tetra chloride (CCl_4) is used to provoke liver fibrosis in mice and rodents. A study investigating the effects of hAEC in CCl_4 injured C57/Bl6 mice, found that following intravenous delivery, cells engrafted and persisted for several weeks in the liver [18]. Similar to effects observed in Bleomycin injured lungs, IL-6, TNFα protein and collagen content declined in the liver. Furthermore, the number of hepatocytes undergoing apoptosis and

number of activated collagen depositing hepatic stellate cells declined significantly in hAEC treated mice [18].

The therapeutic potential of AM derived cells has also been examined in neurological disorders. In particular, stroke has been a major target disease for testing the efficacy of transplantation of AM derived cells. Stroke remains a serious unmet medical condition worldwide and in the US stroke is the primary cause of disability and the third leading cause of mortality. Following the initial stroke episode, inflammation is a major cause of secondary cell death. Although the anti-inflammatory effects of AM derived cells could be beneficial in reducing stroke progression the optimal time and mode of cell delivery need careful assessment. That inflammation may represent a double-edged sword is exemplified in stroke, in that a dynamic modulation of the many inflammatory components in response to ischemic injury is necessary in order to facilitate the functional benefits of cell therapy. For example, the chemokine stromal cell-derived factor-1 or SDF-1, an early pathological inflammatory factor secreted soon after the stroke facilitates the migration of transplanted cells and therefore the early mitigation of SDF-1 may be detrimental. In parallel, stroke may lend a non-conducive brain microenvironment, requiring control of inflammation to some extent to enhance graft survival. To this end, following middle artery occlusion in rats, hAEC and hAMSC were transplanted into the presumed ischemic penumbra (instead of the necrotic core) 2 days after stroke and found to significantly improve motor and neurological deficits by 7 and 14 days and increase the number of healthy host cells within the penumbra [16]. hAEC injection into the penumbra can also lead to reduced infarct size [23]. While direct cell transplantation into the ischemic penumbra is feasible, non-invasive peripheral cell injection may allow a larger patient population to benefit from cell therapy in view of stroke's abrupt onset and rapid progression of debilitating disease symptoms.

In addition to the amniotic cells, the AM membrane itself can exert ameliorative effects and are summarized below.

1.3. Ongoing clinical applications using amniotic membranes

Human AM have a long history in clinical utility. The first application was reported a century ago where the membranes were used as biological dressings to heal skin wounds; a practice that continues to the present day. Currently, AM are also used for treating dermal burns and for open non-healing ulcers and surgical, infected and traumatic wounds [6,24,25]. Since the 1940s, AM have been used in the management of ocular surface disorders. The membrane is used as a graft (with the amniotic epithelium facing outwards) or as a patch (epithelium facing inwards) to cover and repair corneal, conjunctival and limbal defects and surgical incisions made during corrective surgery [26–28]. Further, hAEC and frozen stored AM intact or denuded of the epithelium are being used as feeder layers for the expansion of limbal and corneal stem cells for subsequent transplantation [29]. These ongoing applications led to pre-clinical studies testing the effects of AM on inflammation and fibrosis in lungs and liver.

1.4. Potential innovative applications of amniotic membranes

Recently, small pieces of the entire AM were found to be effective against cardiac ischemia [30] and liver fibrosis [31]. Cardiac ischemia was induced in rats by coronary artery ligation and fragments of AM from human term placenta were applied as patches onto the infarcted myocardium. During the two month follow-up period, treated rats showed improved cardiac dimensions and contractile functions including higher left ventricular ejection fraction, fractional shortening and wall thickening compared to non-treated rats [30].

Liver fibrosis was induced in rats through bile duct ligation (BDL) and AM fragments patched onto the surface of the injured liver [31]. While fibrosis progressed rapidly in controls leading to cirrhosis within 6 weeks of BDL, fibrosis was confined to the portal/periportal regions of the liver in AM-treated rats, without any evidence of cirrhosis and a nearly 50% reduction in collagen deposition [31]. Furthermore, the application of AM significantly delayed the gradual progression of the ductular reaction and reduced the area occupied by activated hepatic myofibroblast cells that deposit collagen [31].

While these initial pre-clinical findings suggests that AM and its' cells may have potential uses in these disease settings, stringent evaluation in larger animal models and comparisons against other cells such as adult bone marrow MSC and hematopoietic stem cells that have also shown to exert beneficial effects and currently being evaluated in clinical trials would be useful. Furthermore, very little is known about the factors that enable survival following xenotransplantation and mechanisms that trigger and lead to the ameliorative effects; some of the potential mechanisms are described below.

1.5. Potential mechanisms involved

A notable feature in the pre-clinical studies outlined above is amniotic cell survival in the absence of overt host responses after xenotransplantation into immunocompetent animals that had not been previously treated with immunosuppressants. Cells that could be transplanted across MHC barriers, without immunosuppression, offer immense scope for wide allogeneic therapeutic applications. hAMSC and hAEC express low levels of HLA Class IA and lack HLA-DR, co-stimulatory molecules CD40, CD80 and CD86 that engage T-cell receptors or are presented indirectly via antigen-presenting cells (APC), to fuel T-cell expansion [1,2,8,13]. One-way lymphocyte reactions have also demonstrated that hAEC and hAMSC fail to induce human T-cell proliferation [32]. Indeed, hAEC have been transplanted into allogeneic volunteers and during trials for lysosomal storage diseases without adverse sequelae attributed to the hAEC [1]. While these studies support the notion that amniotic cells can be transplanted across MHC barriers, generation of antibodies, effects of repeated cell injection as opposed to the possibility of tolerance induction need careful evaluation.

Further, hAEC and hAMSC can modulate immune cell activities. Amniotic cells suppress T-cell proliferation [32,33] with cell–cell contact and trophic factors being likely contributors. Although little is known about the effector molecules responsible, PGE2, TNF-Ra, IL-10, TGFβ and soluble HLA-G from hAEC and/or hAMSC are likely to play a role. hAMSC also inhibit the generation and maturation of APC. In transwell experiments, that only allow passage of soluble factors, hAMSC blocked differentiation and maturation of peripheral blood monocytes into dendritic cells (DC) preventing expression of the DC marker CD1a and reducing HLA-DR, CD80 and CD83 expression [34]. Furthermore, the blockade of monocyte maturation impaired their stimulatory activities on allogeneic T-cells [34]. Investigation into possible mechanism/s showed that hAMSC arrest monocytes in the G0 phase of the cell cycle, abolish TNFα and chemokines CXCL10, CXCL9 and CCL5 whilst greatly elevating the secretion of Th2-related cytokines CCL2, CXCL8 and IL-6 [34]. Effect of hAEC on APC is not known, but hAEC may restrain monocyte migration and activation via MIF-1.

The beneficial effects exerted by AM in the pre-clinical models of myocardial infarction and liver fibrosis, cannot be attributed to cell replacement in the injured tissue. Indeed, no cells derived from transplanted AM were found to have migrated and engrafted into the myocardium or liver. Most likely, the effects observed were associated with the release of soluble factors by cells and molecules

bound to the collagenous stromal matrix of the AM patch that exert paracrine mechanisms to support survival, differentiation and proliferation of host cells. The mechanisms are still undefined, however it has been reported that AM release potent immunomodulatory and anti-inflammatory cytokines (IL-10, IL-6) [35], growth factors associated with wound healing, including angiogenic factors (VEGF, PDGF angiogenin) [36], inducing proliferation (epidermal-, keratinocyte-, hepatocyte- and basic fibroblast growth factors) [37] and differentiation (TGFβ) [36].

The AM was also found to have reduced scarring in the myocardial infarct and livers of animals receiving membrane patches. In ophthalmic investigations it has been shown that hyaluronic acid present in the matrix of the AM can suppress TGFβ and inhibit the differentiation of conjunctival and limbal fibroblasts into myofibroblasts [38]. As TGFβ is a potent pro-fibrogenic cytokine its' reduction can inhibit collagen synthesis. Potentially, similar mechanisms may partly account for the reduction in scarring following the patching of AM. hAEC and hAMSC transplantation was also shown to elicit potent anti-fibrotic effects. Lowering of TGFβ protein was noted in Bleomycin and CCl_4 injured lungs and livers respectively, of mice receiving hAEC, coupled with an induction of collagen degrading matrix metalloproteinases and a reduction of their inhibitors, the TIMP proteins [11,18]. Again paracrine mechanisms induced by factors secreted by the hAEC may be involved. However, while studies show that hAEC are retained for several weeks, cell numbers engrafting are low and decline over time. Further, there is mounting evidence of transdifferentiation of hAEC *in vivo*. Early studies reported differentiation into neural cells, while recent studies report the presence of surfactant protein producing cells in lungs and albumin and α-antitrypsin secreting cells characteristic of hepatocytes in the liver. Whether the growth factor and cytokine milieu of the differentiated cells contribute to inflammation and fibrosis reduction is unknown. A recent study by Tsuji provides insights suggesting that hAMSC differentiating into cardiomyocytes may indeed play such a role [22].

In summary, hAEC and hAMSC have the capacity to differentiate into multiple cell lineages. In addition, the anti-inflammatory and anti-fibrotic effects of these cells and the AM have been demonstrated following transplantation into animal disease models. Ongoing studies relating to safety and efficacy of the transplanted hAEC, hAMSC and AM and mechanisms leading to reparative effects in diseased organs would make a valuable contribution in assessing the true potential of these cells for clinical applications.

Conflict of Interest

The authors state they have no conflict of interest.

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