



Concise Review: Fetal Membranes in Regenerative Medicine: New Tricks from an Old Dog?

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ABSTRACT

The clinical application of the fetal membranes dates back to nearly a century. Their use has ranged from superficial skin dressings to surgical wound closure. The applications of the fetal membranes are constantly evolving, and key to this is the uncovering of multiple populations of stem and stem-like cells, each with unique properties that can be exploited for regenerative medicine. In addition to pro-angiogenic and immunomodulatory properties of the stem and stem-like cells arising from the fetal membranes, the dehydrated and/or decellularized forms of the fetal membranes have been used to support the growth and function of other cells and tissues, including adipose-derived mesenchymal stem cells. This concise review explores the biological origin of the fetal membranes, a history of their use in medicine, and recent developments in the use of fetal membranes and their derived stem and stem-like cells in regenerative medicine. *STEM CELLS TRANSLATIONAL MEDICINE* 2017;6:1767–1776

SIGNIFICANCE STATEMENT

The fetal membranes make up the amniotic sac, which surrounds the fetus during pregnancy. They have nearly a century long history in regenerative medicine. They have been used as biological bandages for skin grafts as well as for serious burns. A variety of stem cells can be isolated from the fetal membranes and their regenerative properties are closely associated to their biological function during pregnancy, which is to protect the fetus from the mother's immune system. We may be able to further exploit their use in regenerative medicine by improving our understanding of the role of fetal membranes during pregnancy.

INTRODUCTION

The interior of the amniotic sac is filled with amniotic fluid, which allows the fetus to move freely in the womb and absorbs physical forces of mechanical injury. The amniotic sac also participates in the metabolism of the fetus, allowing for nutrient transport and contributing to maternal-fetal tolerance. After the delivery of a healthy baby, the fetal membranes are usually discarded as medical waste along with the rest of the placenta. However, the medical applications of fetal membranes have been established for nearly a century. The following concise review will describe the role of the fetal membranes during pregnancy, the stem cells and stem-like cells isolated from the fetal membrane, as well as their applications in regenerative medicine.

IMMUNOMODULATION DURING PREGNANCY

The placenta plays an important role in modulating the mother's immune system during pregnancy.

The placental villi sprout from the chorionic plate are in contact with maternal blood, while branches of the fetal blood vessels carry fetal blood to the villi. During placentation, fetal derived extravillous trophoblasts from the placental villi infiltrate the decidua to remodel uterine spiral arteries in order to achieve an adequate blood supply to the developing fetus [1] (Fig. 1). The implantation site is richly populated by immune cells; ~70% uterine natural killer cells, ~10% T cells, and ~20% myelomonocytic cells [1]. Maternal and fetal immune cells interact directly with each other in the decidua. This interaction is thought to play an important role in fetomaternal tolerance [2]. It is perhaps this evolutionary step in placentation that confers the multitude of beneficial effects associated with gestational tissues and their derived stem cells.

The switch from immune surveillance to immunotolerance is a crucial adaptation during pregnancy. In order to achieve a healthy pregnancy, the maternal immune system must remain quiescent to enable embryo growth. The initial

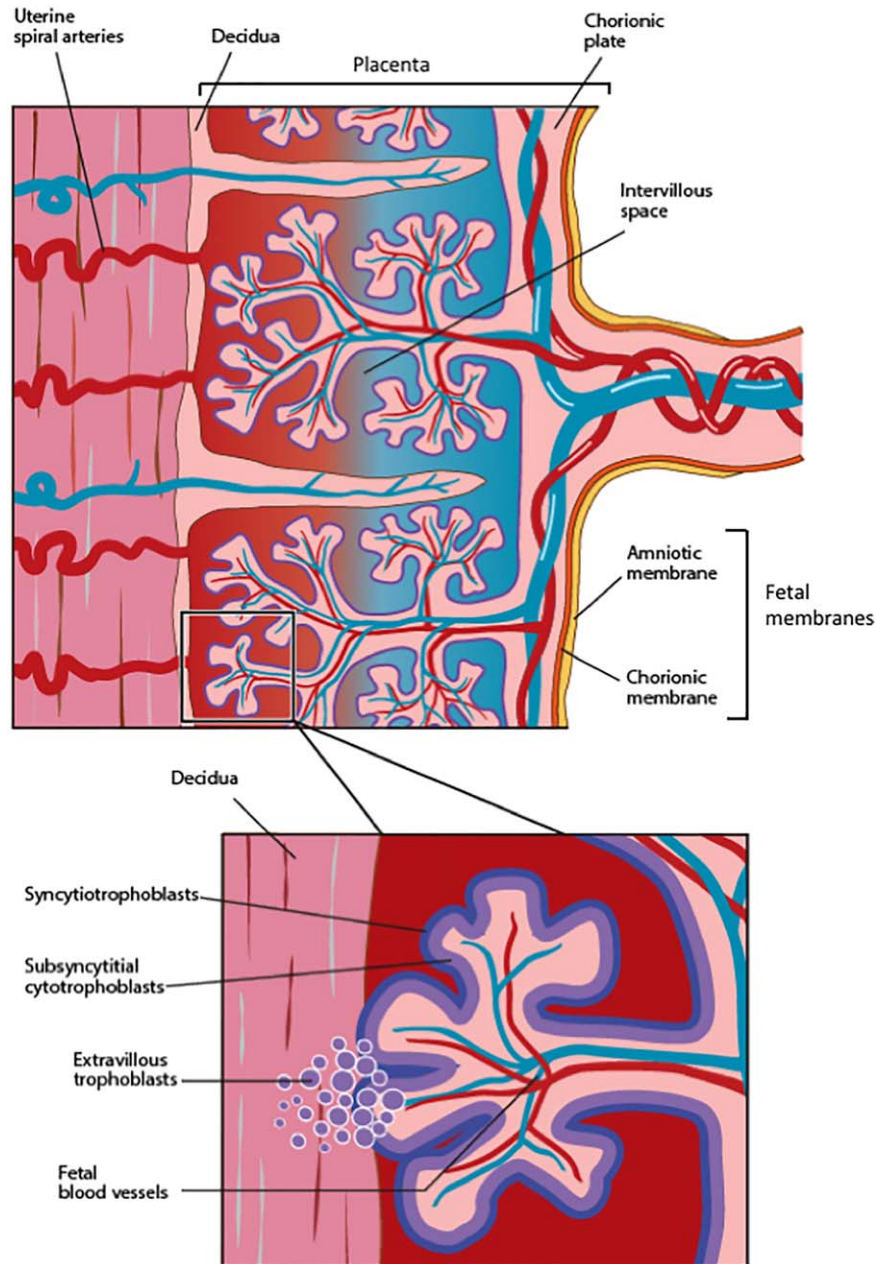


Figure 1. Simplified cross-sectional diagram showing the fetal membranes (i.e., amniotic and chorionic membranes) in relation to the chorionic plate and decidua. The insert shows a representative placental villous tree surrounded by the syncytiotrophoblast layer and its underlying cytotrophoblasts. The extravillous trophoblasts invade the decidua and spiral arteries to remodel the uterine spiral blood vessels such that they become low resistance and thin-walled, enabling a consistent blood supply to the developing fetus.

implantation stage may be seen as an acute, aseptic inflammatory response by the mother's immune system [3]. However, the embryo suppresses this and prevents rejection once it is implanted. This is partly achieved through atypical expression of major histocompatibility complex (MHC) by the trophoblasts and fetal membranes. The fetal membranes themselves express high levels of HLA-G [4], which plays a critical role in maternal-fetal tolerance [5]. The trophoblast serves as an immunological barrier between the mother and the fetus. It is characterized by the lack of MHC class II antigens, [6–8] as well as classical MHC class I antigens—human leukocyte antigen (HLA)-A and HLA-B [9]. This atypical expression of MHC

molecules facilitates fetal tolerance by inhibiting the maternal immune response.

Class II antigens participate in the activation of CD4⁺ helper T (T_H) lymphocytes and consecutively in antibody and cytotoxic T lymphocyte-based immune responses [10]. The absence of class II molecules protects the fetoplacental unit from allogeneic reactions against paternally derived MHC-II antigens, and direct presentation of fetal derived peptides by MHC class II positive trophoblast cells to maternal helper T cells [10]. Pregnancy complications such as spontaneous recurrent miscarriages arise in the absence of these maternal adaptations and when class II antigens are aberrantly expressed [11].

While human trophoblasts do not express the classical HLA-A and HLA-B molecules, they do express classical HLA-C and non-classical HLA-E, -F, and -G class I molecules [10]. HLA-G differs from the other HLA class I molecules in terms of its low polymorphism, its unique promoter region and restricted tissue distribution. Another difference that separates HLA-G from the rest of the HLA class Ia genes, is the alternative splicing of HLA-G mRNA which produces alternative isoforms of the protein [12]. HLA-G can be expressed at the cell surface or as soluble isoforms. The soluble isoforms of HLA-G have been identified at the fetomaternal interface where they appear to modulate maternal-fetal tolerance by inhibiting cytotoxic T cells activity, preventing CD4+ T cell proliferation [13], activating regulatory T cells [14] and inducing CD8+ T cell apoptosis via Fas/FasL [15, 16]. The high expression of HLA-G, and other immunomodulatory molecules such as indoleamine 2,3-dioxygenase, may account for the immune privileged status and immunomodulatory properties of fetal membrane derived stem and stem-like cells.

THE HISTORICAL AND CONTEMPORARY USE OF FETAL MEMBRANES

The fetal membranes and adjacent decidua provide a valuable source of cells with regenerative and immunosuppressive properties that may be useful for the treatment of a variety of inflammatory disorders [17]. Fetal membranes have been used to treat severe burn injuries for almost a century [18]. The fetal membranes comprise the amniotic and chorionic membranes, of which the amniotic membrane (AM) is the innermost layer and is in physical contact with the amniotic fluid. The AM is composed of a monolayer of cuboidal amniotic epithelial cells, which overlay a thick basement membrane, and sparse mesenchymal cells enclosed by an avascular matrix. The amniotic epithelial cells (hAECs) produce numerous cytokines/factors known to promote cell proliferation and differentiation, which appear to remain bound, at least partly, to the extracellular matrix even after de-epithelialization and sterilization of the AM [19, 20].

The first report on the medical use of AM pertained to its application as a skin graft substitute dating back to 1910 [21]. More advanced studies on the possible clinical applications of AM did not begin to gather momentum until the second half of the 20th century where AM became one of the first biomaterials used in tissue engineering—as a biological scaffold to support cell growth and cell migration [22, 23]. For the purposes of ocular surface reconstruction or wound treatment, *in vitro* culture of epithelial cells on AM have been used for graft preparation [24, 25]. The utility of AM has also been extended to thoracic surgery (mainly for surgical closure) [26, 27], reconstruction of ocular surface damage [28, 29] and wound management, especially for severe burns [30, 31] and chronic skin ulcers [32, 33]. It is therefore perhaps unsurprising that stem cells and stem-like cells derived from these tissues retain their wound healing properties.

FETAL MEMBRANE DERIVED STEM CELL POPULATIONS

A variety of stem and stem-like cells can be isolated from term fetal membranes, displaying multi-lineage properties, with abilities to modulate immune responses and release bioactive molecules. The chorion is made up of an inner chorionic mesodermal layer and an outer trophoblastic layer consisting of cytotrophoblast cells

and syncytiotrophoblast of the villi. There are five minimum criteria for defining human amniotic and chorionic mesenchymal stem cells (MSC; hAMSC and hCMSC, respectively), namely:

- fetal origin; $\leq 1\%$ maternal contamination;
- ability to develop fibroblast colony-forming units;
- surface antigen expression of CD90, CD73, and CD105 concurrent with the absence of CD45, CD34, CD14, and HLA-DR;
- multi-lineage differentiation potential; and
- plastic adherence [34]

The existence of a high number of MSCs with osteogenic and adipogenic potential within the amniotic membrane was reported for the first time in 2004 by In't Anker and colleagues [35]. However, fetal membrane derived MSCs appear to have similarly potent immunomodulatory properties as their adipose and bone marrow counterparts [36]. In tissue culture conditions, hAMSC can support the hematopoiesis of CD34⁺ cells in the absence of exogenous cytokines. Cotransplantation of human cord blood-derived hematopoietic stem cells with hAMSC induced an earlier and more complete recovery of hematopoiesis in mice [37]; however, this paradigm has yet to be tested in humans. In addition to this, hAMSC have angiogenic abilities. They spontaneously form capillary-like structures when cultured in semisolid medium, and this was improved by VEGF supplementation [38]. The AM itself is able to support angiogenesis and tissue remodeling, and decrease inflammation by secreting factors including transforming growth factor- β , basic fibroblast growth factor (FGF), epidermal growth factor (EGF), keratinocyte growth factor, and hepatocyte growth factor (HGF) [39]. The hAMSC are able to retain a stable morphology for more than 20 passages with a phenotype that is similar to bone marrow and umbilical cord blood derived MSC (CD29, CD73, CD44 positive and CD14, CD34, CD45 negative) [40].

Transmission electron microscopy of hAMSC show physical characteristics common to both mesenchymal and epithelial cells, and this has been interpreted as a sign of multipotency. This trait is absent in hCMSC, which are more primitive and metabolically quiescent. hCMSC have a simpler cytoplasmic organization with stacks of rough endoplasmic reticulum cisternae, dispersed mitochondria and glycogen lakes. Unlike hAMSC, hCMSC lack assembled contractile filaments, prominence of endocytotic traffic and junctional communications [34]. Notably, fetal membrane-derived stem cells also appear to express numerous cell-surface antigens and intracellular antigens similar to their better characterized counterparts, such as the BM-derived MSC [41]. Fetal membrane derived stem cells express typical mesenchymal markers and stage-specific embryonic antigens (SSEA)-3 and -4, but do not express hematopoietic-, endothelial-, and trophoblast-specific cell markers [38, 42]. However, both hAMSC and hCMSC express low HLA-ABC and no HLA-DR, and their immunoprivileged status likely reflects their embryonic origin [42]. Cultivars of MSC from amnions and chorions present as a homogeneous population of spindle-shaped cells after the first passage. CD105⁺ hAMSC and hCMSC formed a homogeneous layer of fibroblastoid cells following magnetic bead separation, with the ability to differentiate into mesoderm-type cells such as osteoblasts, adipocytes and chondrocytes [43].

The hAEC are another stem-like cell population that has gained recent interest. They can be isolated from the amniotic epithelium, which arises from the epiblast prior to gastrulation, when cell fate is thought to be specified. In contrast, the chorion

differentiates from extra-embryonic trophoblast. It has therefore been speculated that the hAEC may have escaped the specification process that accompanies gastrulation and as such, hAEC may retain some or all of the characteristics of the epiblast including pluripotency. This is reflected in their expression of surface markers and transcription factors characteristic of pluripotent stem cells such as SSEA-3, SSEA-4, TRA-1-60 and TRA-1-81, *Oct4*, and *Nanog* [44, 45]. The hAEC are able to differentiate into cells of the ectoderm, endoderm and mesoderm lineage in vitro [44], with the unique ability to form acetylcholine and catecholamine releasing glial and neuronal progenitor-like cells [46–48]. Similar to MSC, hAEC are able to exert a broad range of immunomodulatory effects including suppression of T cell response, induction of Treg maturation and macrophage polarization [49, 50]. However, the efficacy of hAEC from preterm donors have been found to be inferior to those isolated from term donors [51]. This coincided with lower HLA-G in the hAEC from preterm donors [51], further supporting that beneficial properties of fetal membrane derived stem cells are associated with their roles during pregnancy.

YIELDS AND GROWTH CHARACTERISTICS

The gestational tissues are a rich source of stem cells. For example, hAMSC and hCMSC are easily isolated through mechanical and sequential trypsin and collagenase digestion in significant numbers, $\sim 24 \pm 10$ million hAMSC and 21 ± 14 million hCMSC [52]. Unlike MSC from bone marrow and adipose tissues, expansion of hAMSC and hCMSC in vitro is contentious where some report poor expansion beyond five passages [43], and others report a stable karyotype after 20 passages over a period of 120 days [53]. Serial expansion of stem cells from gestational tissues, albeit with placental MSC, has also been previously shown to be associated with epigenetic alterations when the cells were passaged under serum-free conditions [54]. This may be a cause for concern since the dominant epigenetic changes observed were demethylation of genes associated with ageing and tumorigenesis. However, it should be noted that the reported epigenetic changes did not result in malignant transformation [54]. In light of this, bulk manufacturing protocols for hAMSC and hCMSC with the ability to maintain vigorous growth and stable karyotypes will need to be developed if widespread clinical translation of their pro-regenerative and pro-reparative potential is to be realized. Given recent reports on the importance of MSC priming and impact of biomatrix stiffness on stem function [55–57], these factors should also be given due consideration in protocol development for bulk cultures.

Discrete morphological changes were observed with persistence of only some colonies arising from bulk cultures of hAMSC and hCMSC after 15 passages [52]. This has led to the postulation that isolated fetal mesenchymal cells may contain progenitor cells that only expand under particular culture conditions, including low cell seeding and colony isolation [52]. In contrast, yields of hAEC are 10 times or greater than that of hMSC and hCMSC [44, 58, 59]. Monolayer cultures of hAEC are reactive with antibodies to low-molecular weight cytokeratins, confirming their epithelial nature. However, initially vimentin-negative hAEC can become vimentin-positive during cell culture while remaining positive for cytokeratin [60]. Furthermore, hAEC undergo replicative senescence where they enter a non-dividing state with senescence after 6–10 passages and senescence has been reported to occur even

earlier when plated at lower densities [60]. This has been attributed to integrin-dependent epidermal growth factor receptor (EGFR) activation, EGFR-associated molecular complexes and cell-to-cell interactions, which are facilitated by higher density culture [60, 61]. This is further supported by their maintenance of long telomeres after 5 passages [58].

PRECLINICAL APPLICATIONS

As mentioned previously, stem cells and stem-like cells from the fetal membranes retain their immunomodulatory properties reflective of their biological function during pregnancy, which have been exploited across a broad range of experimental disease models. These are discussed in the following sections and summarized in Table 1.

Cardiovascular Diseases

The angiogenic, cytoprotective, and immunosuppressive properties of hAMSC and hCMSC suggest that these cells, and their secreted soluble factors may be suited for applications for cardiovascular diseases. Their ability to secrete angiogenic factors such as HGF, IGF-1, VEGF, and bFGF were concurrent with their ability to partially rescue experimental critical limb ischemia [62]. The high concentration of PGE2 in hAMSC compared with hCMSC when cocultured with CD4+ T cells suggests that hAMSC may be a better cell source in such settings [62]. These angiogenic and immunosuppressive properties have been exploited in experimental cardiac grafts in rats. Fibrin grafts containing spheroids of subamniotic lining MSC embedded within failing rat hearts resulted in improved cardiac function and revascularization of the ischemic myocardium [63]. Additionally, in vitro cardiomyogenic induction of hAMSCs has been reported to yield spontaneously beating cells with a 33% transdifferentiation efficiency. When transplanted into a rat model of myocardial infarction, they improved impaired left ventricular fractional shortening and significantly decreased the area of myocardial fibrosis [64]. When hAMSC were transplanted into the infarcted myocardium of Wistar rats, cardiomyocyte transdifferentiation was observed in situ, with > 4-week post-transplant survival without immunosuppressants [64]. This apparent tolerance was associated with HLA-G expression, lack of MHC expression, and activation of FOXP3-positive regulatory T cells. Notably, administration of IL-10 or progesterone, which play an important role in fetomaternal tolerance during pregnancy, significantly increased HLA-G expression in hAMCs and increased cardiomyogenic transdifferentiation efficiency [64]. When hAMSC were administered to immunocompromised mice following anterior descending artery ligation, hAMSC improved left ventricular function which correlated with increased myocardial viability and sustained engraftment [65].

The VCAM-1+ subpopulation of hCMSC has been demonstrated to potent vasculo-angiogenic abilities in vitro and in vivo. Conditioned medium from VCAM-1+ hCMSC promoted proliferation and migration on endothelial cells compared with VCAM-1–hCMSCs [66]. Transplantation of VCAM-1+ hCMSCs into the ischemic hind limb of BALB/c nude mice also significantly improved functional outcomes in comparison with VCAM-1– hCMSC [66]. This may therefore suggest that there are subpopulations within each type of fetal membrane derived stem cells with differential therapeutic potentials [66]. In another study, unseparated hCMSC were administered weekly through intramyocardial injections to

Table 1. Summary of preclinical applications of stem and stem-like cells derived from fetal membranes

Disease(s)	Cell type(s)	Reference(s)
Cardiovascular		
Critical limb ischemia	hAMSC, hCMSC	[62]
	hCMSC	[66]
Myocardial infarction	hAMSC	[63–65]
	hCMSC	[67]
Stroke	hAEC	[68, 69]
Neurological		
Traumatic brain injury	hAMSC	[71]
Spinal cord injury	hAMSC	[72]
	hAEC	[76]
Optic nerve crush injury	hCMSC	[73]
Encephalomyelitis	hAEC	[74]
Parkinson's Disease	hAEC	[75]
Diabetes		
	hAEC	[77]
	hAMSC	[78, 79]
	hCMSC	[80]
Gastrointestinal		
Proctitis	hAMSC	[81]
Colitis	hAMSC	[82]
liver fibrosis	hCMSC	[83]
	hAEC	[84, 85]
Respiratory		
Pulmonary fibrosis	hAEC	[49, 50, 108]
	hAMSC, hCMSC	[87]
Chronic obstructive pulmonary disease	hAEC	[91]
Asthma	hAEC	[92]
Bronchopulmonary dysplasia	hAEC	[93, 94]
	hCMSC	[95]
Cystic fibrosis	hAMSC	[96]
	hAEC	[97]

Abbreviations: hAEC, human amnion epithelial cells; hAMSC, human amnion mesenchymal stem cells; hCMSCs, human chorionic mesenchymal stem cells.

immunocompetent C57Bl6 mice following myocardial infarction. After 2 months, hCMSC-treated mice had a significant increase in ejection fraction and a reduction in end-systolic volume without a reduction in infarct size [67]. hCMSC remained in the heart for up to 96 hours after the first injection; however, cell survival was reduced in subsequent injections, thereby suggesting that functional improvement was independent of permanent engraftment [67].

hAEC have been assessed for their potential to modulate inflammation associated with stroke. hAEC transplanted into a rat intracerebral hemorrhagic stroke model improved motor function, and reduced cerebral edema as well as activated microglia, with evidence of transplant survival in the lateral ventricular wall at 4 weeks [68]. Interestingly, induction of epithelial-to-mesenchymal transition by priming with TGF β -1 enhanced the capacity of hAEC to support the ischemic myocardium [69]. hAEC reportedly lost their cobblestone morphology and acquired a fibroblastic

phenotype, with downregulation of E-cadherin, upregulation of N-cadherin, Akt phosphorylation, and intracellular periostin translocation [69]. The mesenchymal-hAEC displayed enhanced cell mobility and secreted gelatinase activity, concurrent with reduced surface presentation of CD105 and CD73, and transcriptional changes that mirrored the loss of epithelial characteristics [69]. When injected intramyocardially into immunocompetent mice after myocardial infarction, global systolic function and longitudinal strain rate was improved in mice that received mesenchymal-hAEC compared to unmanipulated hAEC [69]. These findings indicate that cell priming prior to administration should be investigated as part of the clinical translation process.

Neurological Diseases

The ability of stem and stem-like cells to differentiate into functioning neuronal progenitor-like cells has led many to explore their utility in restoring neurological function. hAMSC were reportedly able to reduce the viability and migratory ability of microglia, and this was associated with production of nitric oxide, which suppresses STAT5 phosphorylation in T cells and promotes immune cell apoptosis [70]. In the setting of traumatic brain injury, transplantation of neuronal progenitors derived from hAMSC improved neurological function and brain histology, with elevated levels of neurotrophic factors (i.e., brain-derived neurotrophic factor, nerve growth factor, neurotrophin 3, glial cell derived neurotrophic factor, and ciliary neurotrophic factor) [71]. Expression of the same factors were observed in hAMSC following neural stem cell differentiation, suggesting that neurotrophic factors released by transplanted hAMSC derived neuronal progenitors may contribute to the improvements seen in experimental traumatic brain injury [71]. In a rat model of spinal cord injury, hAMSC were injected into the contused dorsal spinal cord and transplanted hAMSC migrated into the spinal cord without differentiating into neuronal or glial cells [72]. Compared with the control group, hAMSC transplantation significantly decreased the numbers of activated macrophages/ microglia and apoptotic cells [72]. Transplantation of hAMSC transplantation also significantly increased the levels of brain-derived neurotrophic factor and vascular endothelial growth factor in the injured spinal cord, thus promoting angiogenesis and axonal regeneration [72]. Similarly, hCMSC administered to a rat model of optic nerve crush (ONC) injury increased axonal survival rates, growth-associated protein-43 (GAP-43) and increased expression of hypoxia-inducible factor 1 α [73]. Additionally, ERM-like protein and SLIT-ROBO Rho GTPase activating protein 2 (SRGAP2) were expressed in the optic nerves of the CP-MSC-injected rats with optical nerve crush injury.

The immunomodulatory properties of the hAEC have also been reported in a mouse model of autoimmune encephalomyelitis where hAEC suppressed the development of encephalomyelitis and prevented disease relapse. T cell responses and production of the IL-17A were reduced in hAEC-treated animals. This coincided with a significant increase in the numbers of peripheral T regulatory cells and naïve CD4⁺ T cells as well as Th2 cells in the peripheral lymphoid organs and the central nervous system [74]. The efficacy of hAEC in Parkinson's disease (PD) has also been evaluated. In a rat model of PD with 6-hydroxydopamine lesions, a higher survival rate of dopaminergic neurons was reported, along with an increase in dopaminergic neurons in the substantia nigra and restrained stem cells growth [75]. Transplantation of hAEC into a rat model of spinal cord injury significantly reduced mechanical allodynia [76], suggesting that hAEC transplantation

may be therapeutic for spinal cord injury related neuropathic pain. Interestingly, the protective effects in the setting of experimental spinal cord injury was profound with hAEC than MSC from umbilical cord. hAEC transplantation also modulated activation of astrocytes and microglia in this model of spinal cord injury [76].

Together these reports indicate that stem cells derived from the human fetal membranes are likely to be beneficial for a spectrum of neurological injuries where inflammation is a predominant feature. Given that inflammation progresses in an ongoing continuum, it is likely that the timing of cell administration will be crucial in order to minimize cell death and preserve function. Rapid translation of these biomedical discoveries will require detailed design around these critical therapeutic windows.

Diabetes

A culture protocol was recently developed to grow pancreatic hormone releasing 3D spheroids of hAEC that released C-peptide in hyperglycemic conditions [77]. This may serve as a bridge to clinically useful applications of fetal membrane stem and stem-like cells, which have been shown in experimental models of diabetes to show promise. Transplantation of hAMSC differentiated into pancreatic islet cells, into streptozotocin-induced type I diabetic mice restored bodyweights and normalized hyperglycemia for 7 months [78]. Encapsulation of hAMSC differentiated into insulin producing islet cells in polyurethane-polyvinyl pyrrolidone macrocapsules have also previously been shown to protect the transplanted cells against immune rejection, while normalizing hyperglycemia in diabetic mice [79]. Transplantation of hAMSC derived islet cells into the kidneys of mice with streptozotocin-induced diabetes restored body weight, and normalized the blood glucose levels, which lasted for 210 days. Only human insulin and c-peptide were detected in the blood of normalized mice after 2 months of transplantation, but little mouse insulin and C-peptide. Removal of graft-bearing kidneys from these mice resulted in causing hyperglycemia again [78].

Long-term cultures of hCMSC have been successfully maintained while retaining their ability to differentiate into adipogenic, osteogenic, chondrogenic, and neuronal lineages [80]. hCMSC can also form islet-like cell clusters (ILC) on stepwise exposure to serum-free differentiation media. Gene transcriptional studies indicate that both undifferentiated hCMSC and ILC expressed insulin, glucagon, and somatostatin [80]. However, protein expression of human insulin, glucagon, and somatostatin was only detected in differentiated cells. The differentiated ILC also showed abundance of pancreatic transcription factors *ngn3* and *isl1*, and were able to secrete insulin in response to glucose [80]. Transplantation of undifferentiated hCMSC or differentiated ILC in STZ-induced diabetic mice restored normoglycemia [80].

These studies and others indicate that stem cells from the fetal membranes and adult tissues, such as MSCs derived from adipose tissue and bone marrow, are potentially capable of restoring normoglycemia. These reports are promising and certainly interesting to the scientific community. However, the likelihood for stem cell therapy to replace traditional management of diabetes must be weighed up against current established approaches to diabetes management as well as the emerging area of islet transplantation. Furthermore, long-term biocompatibility and longevity of encapsulation devices remain underdetermined.

Gastrointestinal

The application of fetal membrane derived stem cells in experimental models of gastrointestinal disease has been varied. hAMSC transplantation has been evaluated in radiation proctitis, which is a serious, incurable and common side effect of therapeutic irradiation for intrapelvic cancer [81]. Intravenous administration of hAMSC ameliorated experimental intestinal epithelial attrition associated with radiation injury. This was recapitulated using hAMSC conditioned media [81]. In rats with severe colitis induced by 8% dextran sulfate sodium, hAMSC transplantation significantly ameliorated disease index, weight loss, colon shortening, and histological colitis [82]. Furthermore, mRNA levels of tumor necrosis factor- α , interleukin-1 β , and migration inhibitory factor MIF were significantly decreased in the rectums of hAMSC-treated rats [82].

hCMSC has also been shown to promote liver regeneration. Recently, Hyun and colleagues showed that hCMSC suppressed pro-fibrogenic Hedgehog signaling in a rat model of carbon tetrachloride induced liver fibrosis [83]. Specifically, they showed that hCMSC released extracellular vesicles containing microRNA-125b that target *smo*, a Hedgehog signaling ligand, and this was concurrent with the downregulation of Hh-target genes [83]. hCMSC were also reportedly able to suppress the expression of Hh and pro-fibrotic genes in cocultured LX2 (human hepatic stellate cell) [83].

Similarly, hAEC have been exploited in a necroinflammatory carbon tetrachloride mouse model of liver fibrosis where intravenous hAEC administration reversed established liver fibrosis [84]. This outcome was attributed to their polarization of macrophages from pro-inflammatory M1 to pro-regenerative M2 [84], as well as directly effects on hepatic stellate cells [85]. In the setting of thioacetamide-induced chronic liver failure, intrasplenically transplanted hAEC showed therapeutic efficacy while preserving HLA-G expression [86]. These differentiated hAEC showed evidence of bile canaliculi, albumin secretion, indo-cyanine green elimination, low-density lipoprotein uptake, and inducible CYP3A4 and CYP2C9 enzyme function [86]. These anti-fibrotic and pro-regenerative properties in liver disease have recently culminated in a first-in-man Phase 1b clinical trial for compensated liver cirrhosis (ACTRN12616000437460).

Respiratory Diseases

The anti-fibrotic actions of placental-derived stem cells including those derived from the fetal membranes have been compared in a mouse model of bleomycin-induced lung fibrosis [87]. This study showed that allogeneic and xenogeneic fetal membrane derived stem cells were similarly efficacious in reducing neutrophil infiltration and fibrosis associated with bleomycin instillation [87]. These reports suggest that hAEC, hAMSC and hCMSC may be of use in clinical pulmonary fibrosis [49, 50, 88, 89], particularly given their ability to reverse established lung fibrosis [90]. This approach may also be applied toward chronic obstructive pulmonary disease. In a rat model of cigarette smoke inhalation induced obstructive lung disease, intra-tracheal instillation of hAEC delayed the progression of emphysema and alleviated lung damage by reducing systemic and pulmonary levels of pro-inflammatory cytokine, IL-8 [91]. A recent study in an ovalbumin induced chronic allergic airway disease model showed that hAEC were superior to bone marrow derived MSC in their ability to diminish ovalbumin induced fibrosis and airway hyper-responsiveness [92]. Similarly, hAEC reportedly exerted immunomodulatory effects in the developing lungs such that delivery of hAECs attenuated pulmonary inflammation in sheep fetuses that

were challenged with lipopolysaccharide [93]. In a murine model of hyperoxia induced neonatal lung injury, hAEC also reversed alveolar simplification [94]. When media conditioned by hCMSC from preterm babies were cocultured with fetal rat lungs (E14.5–15.5), *ex vivo* lung growth was markedly accelerated after 72-hour culture [95]. Specifically, Di Bernardo and colleagues observed significant increases in lung surface area, terminal bud formation with a predominant feature of enhanced branching morphogenesis. These outcomes suggest that autologous hCMSC from premature babies may be beneficial for perinatal lung development. However, comparisons of hCMSC from term and preterm donors should be assessed, and their efficacy using *in vivo* models of perinatal lung injury are necessary to evaluate their potential clinical efficacy.

In the context of cystic fibrosis, hAMSCs have been induced to CFTR expressing cells upon isolation and in coculture with CF airway epithelial cells. Freshly isolated hAMSCs initially display low levels of CFTR mRNA, which decreased with serial passaging. When hAMSCs were mixed with CFBE41o- respiratory epithelial cells and seeded onto permeable filters, 33%–50% of hAMSCs acquired CFTR expression on the apical membrane detectable by flow cytometry and confirmed by confocal microscopy [96]. Similarly, hAEC have been reported to form 3D structures that express the CFTR gene and protein following long-term culture in Small Airway Growth Medium (SAGM) [97]. The distribution of CFTR was polarized on the membranes of hAEC cultured in SAGM, similar to that observed in polarized airway cells *in vivo*. Notably, hAEC expressing CFTR also possessed functional iodide/chloride ion channels [97]. These studies indicate that there may be a potential to apply hAMSC and hAEC to cystic fibrosis; however, engraftment of CFTR-expressing cells in sufficient numbers to overcome the genetic defect remains critical to success.

Fetal Membranes for Other Stem Cell Types

It is interesting to note that fetal membranes have also been used to support the growth of other stem cell types. Dehydrated fetal membranes consisting of both the amnion and chorion have been shown to support the proliferation and wound healing potential of adipose-derived stem cells from diabetic patients [98]. When implanted under the skin in a cutaneous ischemia model, the fetal membranes recruited circulating hematopoietic progenitor cells to sites of neovascularization [99]. These findings have led some to postulate that dehydrated whole fetal membranes may be an alternative approach to regenerative medicine.

Human adipose-derived mesenchymal stem cells (hADMSCs) seeded onto radiosterilized human amnion have been tested in their ability to aid cutaneous wound repair in the form a biological bandage. In a study by Sánchez-Sánchez and colleagues, AM were used as a biological scaffold for the culture and maintenance of hADMSCs, where the membranes were found to induce hADMSC to secrete IL-10 as well as IL-1 β , whose interplay is thought to be vital for a balanced restoration of all necessary tissues [100]. Decellularized AM has also been found to be suitable as a scaffold for the delivery of hADMSC in tissue engineering and regenerative medicine applications [101]. Sheets of cultured dental pulp MSCs prepared for periodontal tissue repair, using AM as a scaffold, retained their stem cell phenotype while promoting cell proliferation [102].

Other Applications

The fetal membranes have been used in other applications such as their utility as a feeder layer for human embryonic stem cell derivation and maintenance [103]. Air-dried and freeze-dried amniotic membranes (AM) have been assessed for their feasibility as a growth substrate for chondrocyte expansion, and were found to improve chondrocyte proliferation, GAG expression and attachment than monolayer cultures [104]. In their lyophilized state, decellularized AM showed potential as a novel barrier for guided bone regeneration in large bone defect where the membrane could afford protected space for osteogenesis while concurrently protecting against fibroblast invasion [105]. Furthermore, an extract of AM has been postulated as an anti-hemolytic and anti-thrombotic due to its abundance in glycosaminoglycans (i.e., perlecan and hyaluronic acid) which inhibit coagulation, as well as as IL-10 and MMP-9 which inhibit platelet aggregation [106].

CONCLUSION

The fetal membranes have been used in regenerative medicine for nearly a century—long before the term “regenerative medicine” was coined [107]. It is now clear that new uses for the fetal membranes, as well as the stem and stem-like cells derived from the membranes, are being discovered. Fetal membrane derived stem and stem-like cells are already being translated into early phase clinical trials. The publication of long-term safety outcomes from these trials will significantly shape their broader application in years to come. Looking ahead, one has to also consider how best to design later phase clinical trials in order to ascertain the ideal therapeutic dose and dosing regimen, as well as route and timing of cell administration. It is also important to improve our current understanding on the biological role of fetal membrane derived stem and stem-like cells in pregnancy and fetal wound healing responses. By understanding their microenvironment as they exert their potent pro-regenerative effects, we can better develop cell priming protocols and design bioreactors that will retain their efficacy in a cost-effective manner for large scale clinical use. It is also clear that the unique mechanical and biochemical properties of the fetal membranes lend themselves to bioengineering applications. Regardless of whether they are used as biological scaffolds for the propagation and maintenance of other cell types or used on their own to repair surgical defects, it is evident that fetal membranes may serve a multitude of clinical purposes. In summary, the field of regenerative medicine is becoming increasingly aware of the usefulness of the fetal membranes. While the use of fetal membranes in medical practice is not new, we are certainly seeing increased novel applications of these membranes and the cells derived from them.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

The author indicated no potential conflicts of interest.

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