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Fetal membranes as a source of stem cells

Kmiecik G[1,*], Niklińska W[1], Kuć P[2], Pancewicz-Wojtkiewicz J[1], Fil D[1], Karwowska A[3], Karczewski J[3], Mackiewicz Z[1]

1 Department of Histology and Embryology, Medical University of Bialystok, Bialystok, Poland
2 Department of Perinatology, Medical University of Bialystok, Bialystok, Poland
3 Department of Hygiene and Epidemiology, Medical University of Bialystok, Bialystok, Poland

* CORRESPONDING AUTHOR:
Department of Histology and Embryology,
Medical University of Bialystok
Waszyngtona 13
15-269 Bialystok, Poland
Tel./Fax: +4885 748 5455
e-mail: gabriela.kmiecik@umb.edu.pl (Gabriela Kmiecik)

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ABSTRACT

In recent years, a constant growth of knowledge and clinical applications of stem cells have been observed. Mesenchymal stromal cells, also described as mesenchymal stem cells (MSCs) represent a particular cell type for research and therapy because of their ability to differentiate into mesodermal lineage cells. The most investigated source of MSCs is bone marrow (BM). Yet, collection of BM is an invasive procedure associated with significant discomfort to the patient. The procedure results in a relatively low number of these cells, which can decrease with donor´s age. Therefore, it seems to be very important to find other sources of mesenchymal stem cells nowadays. A human placenta, which is routinely discarded postpartum, in spite of its natural aging process, is still a rich source of stem cells capable to proliferate and *in vitro* differentiate in many directions. Besides homing and differentiation in the area of injury, MSCs there elicit strong paracrine effects stimulating the processes of repair. In this review, we focus on the biology, characteristics and potential clinical applications of cells derived from human fetal membranes: amnion and chorion.

Key words: : Mesenchymal stromal cells, amniotic epithelial cells, amnion, chorion, differentiation, paracrine effects

INTRODUCTION

Stem cells biology has become one of the most interesting and most often studied subject, especially in the context of regenerative medicine. The use of stem cells in the regeneration, repair or replacement of damaged tissues and organs is currently the subject of many studies [1-3]. In particular, in the context of tissue engineering and regenerative medicine, BM-isolated mesenchymal stromal cells/mesenchymal stem cells (MSCs) are of great interest. The first report on the presence of nonhematopoietic stem cells in BM was proposed by German pathologist Cohnheim about 130 years ago. Further research conducted by Friedenstein *et al*. [4,5] have proved that the BM is the source of subpopulation of fibroblast-like cells capable of adherence to tissue culture plastic, colony forming unit (CFU) capacity, differentiation into fibroblasts and other cells of mesodermal origin. MSCs can give rise to progenitor cells of osteoblasts, chondroblasts, adipocytes, cardiomyocytes and skeletal muscles. Multipotent plastic-adherent cells isolated from BM and other tissues should be currently termed mesenchymal stromal cells, often referred as mesenchymal stem cells [6]. Multipotential character of MSCs enables their use in many areas of regenerative medicine. Due to the BM harvesting limitations, alternative sources of MSCs have been sought. The presence of MSCs has been demonstrated in many tissues, also in fetal membranes.

REVIEW

Stem cell hierarchy

The notion of 'stem cells' refers to various types of cells, which are unspecialized, undifferentiated cells capable of generating one or more cell lineage types of the germ layer, and also have the ability to self-renewal. Moreover, these cells have a great capacity to differentiate towards different types of mature cells. Based on the differentiation potential stem cells can be classified into totipotent, pluripotent, multipotent and unipotent stem cells. Several varieties of human stem cells have been isolated and identified. The most primitive stem cell, during human embryo development, presenting totipotent potential is zygote or first blastomers, which are formed with the first division of the zygote. These cells can give an origin to complete embryo and trophoblast development. During subsequent divisions morula is formed, which has lost totipotential character. These cells are pluripotent capable of forming tissues originating in the three germ layers, but they have lost the ability to form the trophoblast. A fully developed blastocyst contains a group of cells called the inner cell mass, which are pluripotent and can give rise to all three germ layers [7,8]. More specialized are multipotent stem cells that can differentiate into a number of cells, but derived from the same germ layer. Multipotent stem cells give rise to unipotent stem cells that can generate a single cell type characteristic for various tissues and organs [9]. Generally, depending on the origin, stem cells have been divided into four main groups: embryonic stem cells, fetal stem cells, perinatal stem cells and adult stem cells. Embryonic stem cells (ESCs) are an example of totipotent cells that can develop all the tissues of the fetus. However, the use of embryonic stem cells is subjected to ethical and social considerations. In addition, because of unlimited ability to proliferate, the potential risk of malignancy is higher in comparison to other types of human cells. Fetal tissues, as blood, kidney, liver, lung are an opulent source of human stem cells, but their application also presents the ethical objections. Therefore, the use of adult stem cells is increasingly important. Adult stem cells can be isolated from several sources, as BM, blood, skeletal tissue, adipose tissue, liver, skin and dental pulp [10-14]. One of the most investigated example is BM, where hematopoietic and non-hematopoietic stem cells can be found [15]. BM contains many different kinds of stem cells, which are used for organism self-regeneration [14,16]. However, the presence of multipotential mesenchymal stromal cells in BM has been described by many researchers, it should be noted that the cultures of MSCs can be occasionally contaminated by pluripotent/multipotent stem cells found in bone marrow [6,15,17]. BM harbors endothelial stem cells (ESCs), multipotential adult progenitor cells (MAPCs), mesenchymal stem cells (MSCs), marrow-isolated adult multilineage inducible cells (MIAMIs), very small embryonic-like stem cells (VSELs) [15,17-20].

Mesenchymal stem cells/mesenchymal stromal cells

Mesenchymal stem cells, also called as mesenchymal stromal cells, are currently being investigated by many researchers as potential therapeutic agents [21,22]. MSCs are a promising cell source for tissue engineering and cell-based therapeutics because of their ability to self-renew and differentiation into specific functional cell types [23,24]. MSCs are defined as cells capable of expansion, self-renewal and differentiation at least to osteocytic, chondrocytic and adipocytic lineages after stimulation [25]. Due to the recent progress in stem cell biology, the number of tissues with the potential for tissue engineering is constantly increasing [26]. MSCs have been isolated from several tissues, including BM [14], adipose tissue [27-31], dental pulp [32], skin [31,33,34], peripheral blood [35], umbilical cord blood [36-39], amniotic fluid [40-42], amniotic membrane [43,44], placenta [25]. Traditional source of MSCs for clinical investigations is BM. Extensive studies of BM-derived MSCs (BM-MSCs) have proven their multipotent differentiation potential and powerful immunosuppressive qualities [45]. However, the collection of BM is associated with invasive procedure involving significant discomfort to the patient. Moreover, it results in a relatively low amount of MSCs (approximately 0.001-0.01% of all isolated nuclear cells) in adult human BM, and the number of cells decrease with donor´s age [14,46]. MSCs are also present in fetal organs, such as liver, BM, kidney, and circulate in the blood of fetuses, but their use is subjected to ethical considerations [14,28,29]. Searching for easily accessible and high-yielding source of stem cells have led many investigators to focus on human placenta. Successful formation of human placenta which plays a crucial role during embryo development, may also represent a reserve of undifferentiated cells. Stem cells isolated from human term placenta represent many advantages. First of all, non invasive procedure is required to obtain the organ. Moreover, there are no ethical objections, because the placenta is routinely discarded postpartum. Despite of the natural aging process of this organ, postpartum placenta still remains a valuable source of stem cells.

Human fetal membranes

Human placenta is composed of fetal component, the chorionic plate and maternal component–deciduas. The chorionic plate consists of connective tissue and forms the wall of the amniotic cavity, it contains chorionic arteries and veins. Chorionic plate is formed by amnion and chorion strictly adhering to each other. On the perimeter amnion and chorion form amniotic sac filled with amniotic fluid, providing and protecting fetal environment. The inner layer, amnion consists of epithelial and stromal layers. The first one is ectodermally derived epithelium uniformly arranged on basement membrane, that is one of the thickest membranes in human organism. The second one is collagen-rich mesenchymal layer, originated from extraembryonic mesoderm, and can be divided into

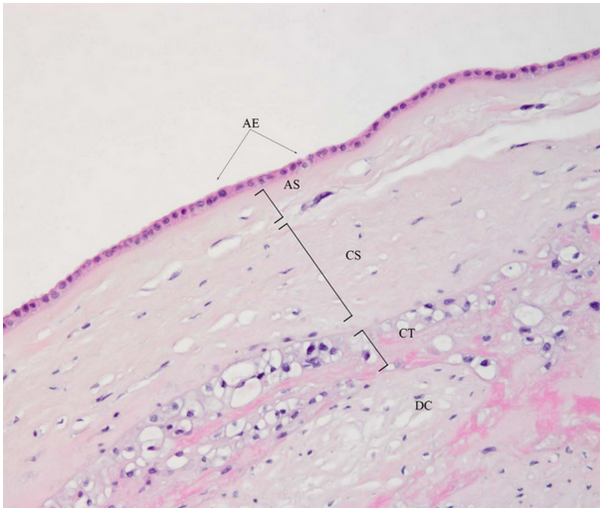
the compact layer, fibroblast layer and an intermediate layer, also called the spongy layer or *zona spongiosa*. Chorion is the outer membrane surrounding fetus, composed of trophoblastic chorionic and mesenchymal tissues. During enlargement of amniotic cavity, the amnion and chorion loosely fuses into single amniochorionic membrane seen after delivery [47]. Fetal placenta tissue cell populations consist of human amniotic epithelial cells (hAECs), human amniotic mesenchymal stromal cells (hAMSCs), human chorionic mesenchymal stromal cells (hCMSCs), and human chorionic trophoblastic cells (hCTCs).

Human amniotic epithelial cells hAECs

The hAECs forms a monolayer of ectodermally derived epithelium uniformly arranged on the basement membrane (*Fig. 1*), which stay in constant contact with amniotic fluid. The epithelial nature of hAECs was confirmed by the presence of epithelial markers cytokeratin 1, 2, 3, 4, 5, 6, 7, 8, 10, 13, 14, 15, 16 and 19. Recent reports indicate that hAECs express stem cell markers and have the ability to differentiate into all three germ layers [48]. After isolation hAECs express very low levels of human leukocyte antigen (HLA) – A, B, C. During cultures, after passage 2 higher levels HLA antigens are observed. Cell surface antigens characteristics for hAECs are ATP-binding cassette transporter G2 (ABCG2/BCRP), CD9, CD24, E-cadherin (CD324), integrins α6 and β1, c-met (hepatocyte growth factor receptor), stage-specific embryonic antigens (SSEAs) 3 and 4, and tumor rejection antigens (TRAs) 1-60 and 1-81. Surface antigens which seems to be absent on hAECs are SSEA-1, CD34, CD133. CD117 (c-kit) is either negative, or may be expressed on at low levels. Although CD90 (Thy-1) is expressed on freshly isolated cells at low levels, but the expression of this antigen increases significantly in culture [49]. Moreover, the presence of epithelial (e.g. E-cadherin, CK7, CD49f, EpCAM) and MSC markers (CD44, CD105, CD146) varies during cultures. hAECs at P0 present the typical epithelial markers, whereas P5 hAECs show expression of CD44, CD105 and CD146 [50]. Surface markers are presented in *Tab. 1.*

In addition to surface markers, hAEC express molecular markers of pluripotent stem cells, including octamer-binding protein 4 (OCT-4), SRY-related HMG-box gene 2 (SOX-2), and Nanog. This suggests that hAECs may be pluripotent [48,49].

Figure 1. **Hematoxylin and eosin stained human term placenta. The amnion is composed of amniotic epithelium (AE) and amniotic mesenchymal stromal layer (AS). The chorionic membrane consist of a stromal layer (CS) and chorionic trophoblast cells (CT). Under the chorion maternal decidual cells (DC) are present. Original magnification x 200.**



Mesenchymal stromal cells from amnion and chorion

According to criteria proposed by Dominici *et al.* [6], for BM-MSCs, mesenchymal cells isolated from amnion and chorion should be defined as mesenchymal stromal cells. *Tab. 2* presents minimal criteria for defining hAMSCs and hCMSCs. Human amnion mesenchymal stromal cells (hAMSCs) are derived from embryonic mesoderm. These fetal cells express low levels of the major histocompatibility complexes (MHC) class I and MHC class II antigens on their surface. Like BM-MSCs, both hAMSCs and hCMSCs adhere and proliferate on tissue culture plastic. These cells present characteristic

Table 1. **Specific antigens expressed on human amniotic epithelial cells (hAECs), human amniotic mesenchymal stromal cells (hAMSCs) and human chorionic mesenchymal stromal cells (hCMSCs).**

Cell type	Phenotype	References
hAECs	Mesenchymal and embryonic markers: CD90+, CD105+, CD73+, CD44+, CD166+, CD29+, HLA-A,B,C+, CD13+, CD24+, SSEA-3+, SSEA-4+, TRA-1-60+, TRA-1-81+, NANOG+, SOX2+, SSEA-1-, CD117 (+/- very weak signal), CD49e- Hematopoietic markers: CD34-, CD45-, CD14-, CD11-, HLA-DR-, CD31- Others: CD324+, CD349-	[44, 48, 49, 50, 51, 52, 53]
hAMSCs	Mesenchymal and embryonic markers: CD90+, CD105+, CD73+, CD44+, CD166+, CD29+, HLA-A,B,C+, CD13+, CD49d+, CD49e+, CD54+, Oct-3/4+ Hematopoietic markers: CD34-, CD45-, CD14-, CD31-, HLA-DR-, CD133-, CD3- Others: CD349+, CD140b+, CD324-	[43, 44, 51, 53, 54, 55, 56, 57, 58]
hCMSCs	Mesenchymal and embryonic markers: CD90+, CD105+, CD73+, CD44+, CD166+, CD29+, HLA-A,B,C+, CD13+, CD10+, CD49e+, CD54+, SSEA-4-/+, NANOG+, SOX+, CD117- Hematopoietic markers: CD34-, CD45-, CD14-, CD31-, HLA-DR-, CD3-, CD133- Others: CD349+, CD140b+, CD324-	[43, 51, 53, 59]

Table 2. **Minimal criteria for defining human amniotic mesenchymal stromal cells (hAMSCs) and human chorionic mesenchymal stromal cells (hCMSCs).**

A specific pattern of surface antigen expression:	
CD90 CD73 CD105	CD45 CD34 CD14 HLA-DR
positive cells (≥95%)	negative cells (≤2%)
Adherence to plastic	
Formation of fibroblast colony-forming units	
Differentiation potential toward one or more lineages, including osteogenic, adipogenic, chondrogenic, vascular/endothelial	
Fetal origin	

fibroblast-like or spindle-like appearances, form clonal colonies and express the typical range of BM-MSC associated cell surface antigens. Moreover, these cells can be induced *in vitro* to differentiate into mature cell lineages. hAMSCs and hCMSCs express typical mesenchymal markers: CD90, CD105, CD73, CD166, CD49e, CD44, CD29 and CD13, but they are negative for hematopoietic (CD31, CD34, CD45) and monocyte (CD14) markers [60].

Isolation and cultivation of cells from fetal membranes

Cells from amnion and chorion can be isolated easily, and different methods of cells isolation have been published [43,44,49,51,61]. In order to isolate cells from human term placenta, amnion and chorion are separated by mechanical detachment. Separation is facilitated by the elastin lamina present in the loose connective tissue of the amnion. Amniotic membrane can be a source of two different types of cells, both having stem-cell characteristics.

hAECs are obtained after removal of the epithelial layer of amnion. It is performed with a digestion with trypsin, dispase or other digestive enzymes, in different concentrations and for different periods of time. Second population of cells– hAMSCs can be gained by a two-step procedure: minced amnion tissue is treated with trypsin to remove hAECs, and the remaining mesenchymal cells are then released by digestion with collagenase or collagenase and DNase. hAECs are small-size cells that are easy to expand *in vitro* cultures for at least 3 passages without morphological changes, but cells do not proliferate in a low density. They grow in a lattice and represent a typical cuboid morphology of epithelial cells. Generally, they present a central or eccentric nucleus, one or two nucleoli and abundant cytoplasm, usually vacuolated. The hAMSCs cells have a fibroblast-like or spindle-shape cell morphology typical for mesenchymal stem cells isolated from BM. They can be simply expanded *in vitro* for at least 9 passages without significant changes in cells morphology. Both hAECs and hAMSCs grow in Dulbecco´s modified Eagle´s media (DMEM) supplemented with 10-20% fetal bovine serum (FBS) and 1% penicillin-streptomycin seeded into culture flasks or dishes. These populations should be cultivate in a humidified 5% CO_2 atmosphere at 37°C. In order to demonstrate the purity of isolated cells populations it is recommended to perform immunohistochemical staining for cytokeratin 7 (CK7), and only hAECs should be positive for this epithelial markers. There are contradictions with the number of passages at which hAECs and hAMSCs stop to proliferate. Miki *et al.* [49] and Parolini *et al.* [61] state that hAECs grow rapidly for 2 to 6 passages before proliferation ceases. On the other hand Diaz-Prado *et al.* [44] indicate that both hAECs and hAMSCs maintain characteristic phenotypes from passages P0 to P9. Moreover, Portmann-Lanz *et al.* [51] have showed that cells isolated from amniotic and chorionic mesenchyme underwent cell death after fourth or fifth passage. On the contrary, Soncini *et al.* [43] confirmed in their observations that hAMSCs and hCMSCs can be cultured *in vitro* at least 15 passages without morphological alterations, but they studied cells at P4 to cells characterization and assessment of multilineage potential.

Human chorionic mesenchymal stromal cells (hCMSCs) are isolated from chorion after mechanical and enzymatic removal of the trophoblastic layer with dispase. Chorionic mesodermal tissue is then digested with collagenase or collagenase plus DNase. Contaminating decidual cells can be present if mechanical dissection is insufficient and fetal genotyping may be needed to evaluate the purity of isolated cells. hCMSCs have also been isolated from chorionic fetal villi through explant culture, but maternal contamination is more likely. Many reports have shown, that placenta-derived cells, including hCMSCs, are able to survive during culture for 10 passages without significant morphological changes [60,62].

Differentiation potential

The differentiation of hAECs has been investigated extensively *in vitro*. Both primary and cells at first passage differentiate into lineages derived from ectoderm (neurons, astrocytes, glia), mesoderm (osteocytes, adipocytes, cardiomyocytes, myocytes) and endoderm (hepatocytes, pancreatic cells) [49,51,52,63,64]. It suggests, that hAECs have a pluripotent character and can give rise into cells of all three germ layers. Ilancheran *et al.* [52] and Wei *et al.* [65] described differentiation of hAECs into classical mesodermal lineages cells as myocytes, osteocytes, chondrocytes and adipocytes.

The differentiation of hAECs into cardiac cells was firstly investigated and described by Miki *et al.* [49]. They showed by RT-PCR that cardiac-specific genes atrial and ventricular myosin light chain 2 (MLC-2A and MLC-2V) and the transcription factors GATA-4 and Nkx 2.5 were expressed in hAECs cultured in the presence of ascorbic acid for 2 weeks. The immunohistochemical analysis of α-actinin

expression was similar to the one reported for hESC-derived cardiomyocytes [49].

hAECs express some differentiation markers for neural stem, neuron and glial cells such as nestin, GAD (glutamate decarboxylase), GFAP (glial fibrillary acidic protein), CNP (cyclic nucleotide phosphodiesterase) [49]. Kakishita *et al.* [66] found that human amniotic epithelial cells differentiate into neural cells (ectodermal lineage) which also synthesize *in vitro* and release catecholamines such as dopamine (DA). This suggests their potential use in the treatment of neural degenerative disorders, e.g. Parkinson's disease.

Under specific conditions hAECs differentiate into hepatic-like cells what was demonstrated by Sakuragawa *et al.* [67]. These authors indicated that cultivated hAECs produced albumin and α-fetoprotein. Further studies demonstrated that these cells present other features associated with hepatocytes, such as glycogen storage and expression liver-enriched transcription factors, e.g. hepatocyte nuclear factor (HNF) 3γ and HNF4α, CCAAT/ enhancer-binding protein (CEBP α and β) and drug metabolizing genes (cytochrome P450) [49,68,69].

Differentiation of hAECs into pancreatic cells (endodermal lineage) has been also investigated. Miki *et al.* [49] showed by RT-PCR analysis, that freshly isolated hAECs expressed pancreas duodenum homeobox-1 and the mRNA expression was maintained when the cells were cultured in the presence of nicotinamide. The expression of the early pancreatic transcription factor PDX-1 and the downstream transcription factors Pax-6 and Nkx 2.2 and the mature hormones insulin and glucagon were identified after 14 days of culturing with media supplemented with nicotinamide.

Mesenchymal stromal/stem cells from various parts of human placenta have been shown to differentiate into chondrogenic, osteogenic, endothelial, hepatocytic and myogenic lineages, but presenting differences depending on the origin of the cells. Both hAMSCs and hCMSCs differentiate toward 'classic' mesodermal lineages (osteogenic, chondrogenic, adipogenic). Moreover, differentiation of hAMSCs to all three germ layers–ectoderm (neural), mesoderm (skeletal muscle, cardiomyocytic and endothelial) and endoderm (pancreatic) – has been described [51,56,57,64,65,70-74]. Chondrogenic differentiation was investigated by Soncini *et al.* [43] by incubating cells for 2-3 weeks in DMEM low glucose containing dexamethasone, L-ascorbic acid 2-phosphate, sodium pyruvate, proline, ITS (insulin, transferrin, selenous acid) and TGF-β1 in appropriate concentration. The ability to undergo chondrogenic differentiation was assessed by toluidine blue staining, which demonstrated cartilage-specific metachromasia in comparison to the cells cultured in control medium [43]. Also osteogenic and adipogenic differentiation of hAMSCs was presented by Wang *et al.* [58]. For osteogenic differentiation cells were stimulated for 14 days in DMEM supplemented with 10% FBS, dexamethasone, sodium β-glycerophosphate and ascorbic acid-2-phosphate. For adipogenic differentiation cells were incubated in adipogenic medium consisted of DMEM with 10% FBS, dexamethasone, indomethacin, 3-methyl-1-isobutylxanthine and insulin. In order to confirm osteogenic differentiation, calcium deposits were analyzed using Alizarin red staining. After 14 days of adipogenic differentiation cells were stained with Oil Red O to evaluate accumulation of lipid-rich vacuoles [58].

Portmann-Lanz *et al.* [51] demonstrated the mRNA expression of myogenic transcription factors such as Myo D and Myogenin and the protein expression of desmine which confirmed the ability of hAMSCs to myogenic differentiation. Alviano *et al.* [56] confirmed the potential of myogenic differentiation of hAMSCs and was the first one, who demonstrated angiogenic potential of hAMSCs. Their experiment indicated that hAMSCs cultured in presence of VEGF expressed endothelial-specific markers such as the receptors of the vascular endothelial growth factor 1 and 2 (FLT-1, KDR), ICAM-1 and also manifestation of CD34 and von Willebrand Factor (vWF) positive cells [56].

Additionally, cardiomyogenic potential has been showed by Zhao *et al.* [75]. They demonstrated that hAMSCs stimulated with bFGF or activin A expressed Nkx2. – a cardiac-specific transcription factor–the earliest marker of heart precursor cells in all vertebrates, and ANP (atrial natriuretic peptide), which is also a cardiomyocyte-specific gene expressed in ventricular myocytes *in vivo*. Also the potential of hAMSCs to differentiate into hepatocytes was investigated [72]. To induce differentiation into hepatocytes cells were cultured in α-MEM supplemented with 10% FBS, human hepatocyte growth factor (hHGF), human fibroblast growth factor-2 (hFGF-2), oncostatin M (OSM) and dexamethasone. After 3 weeks immunofluorescence analysis presented induction of the expression of albumin and α-fetoprotein. Furthermore, the storage of glycogen in hAMSCs following their differentiation into hepatocytes was observed.

In 2008, Tamagawa *et al.* [76] described differentiation of human amnion-derived fibroblast-like cells into neural-like cells. In their previous study, the cell populations obtained after enzymatic digestion with trypsin-EDTA, collagenase, dispase and papain were designated as mesenchymal cells derived from human amniotic membrane. However, they showed that these cells are not simply mesenchymal cells as they can also differentiate into endoderm-derived hepatic cells, so they re-designated these cells as human amnion-derived fibroblast-like cells. After induction of neural cell differentiation the expression of neuron-specific genes, such as neuron specific enolase (NSE), neurofilament-medium (NF-M), β-tubulin isotype III (TUJ1), and glial fibrillary acidic protein (GFAP) were analyzed. The expression levels after induction of neural cell differentiation were abundantly higher in comparison to levels of expression before differentiation [76].

Two different types of primitive cells may be obtained from human chorion: chorionic mesenchymal stem/stromal cells (hCMSCs) and chorionic trophoblastic cells (hCTCs) [67]. hCTCs haven't been extensievely examinated as well little reports of these cells have been published. hCMSCs present multipotential character capable of differentiation into chondrocytes, osteocytes, adipocytes, myocytes as well as neuron-like cells and present comparable or even greater differentiation potential than amnion-derived mesenchymal cells [43,51,59,60]. It can be associated with different origins of both membranes: the chorion is derived from the trophoblast, while the amnion arises from the embryoblast. Despite the significant differentiation potential displayed by these cells, the number of experiments with hCMSCs is limited, probably due to their limited survival in advanced passages. Jones *et al.* [59] compared first trimester- and term fetal placental chorionic-derived stem cells considering their phenotype, growth kinetics and differentiation potential. They indicated that first trimester isolated cells shared a common phenotype with term placental cells. Both types of cells differentiated into osteogenic, adipogenic and neurogenic pathways. However first trimester isolated cells present features of earlier stage of stemness, such as smaller size, faster kinetics, expression of OCT4A variant 1 and greater expression levels of NANOG, SOX2, c-MYC, KLF-4. Moreover, transplantation of these cells into *osteogenesis imperfecta* mice improved bone quality and plasticity compared to term placenta isolated cells [59]. Many studies of human term placenta report that isolated cells display multipotential character. However, some reports refer to both, fetal and maternal origin of cells [44,54], as well as only a maternal origin [77,78]. Furthermore, cells isolated from human term placenta are termed as placenta-derived mesenchymal stem cells (PD-MSCs) by some authors. PD-MSCs also differentiate *in vitro* into derivatives of the mesenchymal cell lineage such as chondrocytes, osteocytes, myocytes and adipocytes [51,62,79-81]. Besides hepatocyte-like cells and neural-like cells differentiation of PD-MSCs has been demonstrated [82-84].

Immunomodulatory properties

One of the advantages of cells derived from fetal membranes, that makes them useful in stem cell based therapies, is their low immunogenicity. hAECs, hASCs, hCMCs lack or present very low expression of highly polymorphic HLA class I antigens (HLA-A, B, C) and nearly no MHC class II (HLA-DP, DQ, DR) on their surface [49,51,52]. These cells also do not express co-stimulatory molecules, such as CD40, CD40 ligand, CD80 and CD86 [53,85,86]. Cells from amnion, chorion and PD-MSCs exert immunosuppressive effects *via* direct suppression of T and B lymphocytes proliferation induced by mitogens or alloantigens, often in a dose-dependent manner [87-91]. These cells can also secrete cytokines engaged in angiogenesis, tissue repair or immune modulation, e.g. VEGF, IL-6, IL-11, M-CSF.

Engraftment of amnion and chorion derived cells in xenogenic models may lead to avoidance or even active suppression of host immune response. Bailo *et al.* [90] confirmed that fetal membranes derived cells fail to induce allogenic and xenogenic lymphocyte responsiveness. Amniotic membrane is commonly used for transplantation to induce epithelialization in burns and skin ulcerations, as well as a dressing for wounds or skin grafts [92-94]. Fragments of amniotic membrane is also extensively used in the treatment of ocular surface reconstruction [95-97]. Amniotic epithelium and amniotic membrane stroma is a source of epidermal growth factor and keratinocyte growth factor which promote wound healing. Furthermore, presence of laminin and type VII collagen fibers in the basement membrane of amniotic membrane are the basis for the observed epitheliotropic effects [98,99]. Their low immunogenicity and anti-inflammatory properties allow for using them as an alternative material in the field of regenerative medicine.

Paracrine effects

The use of MSCs for tissue repair was initially based on the expectation that these cells are able to home and differentiate within the damaged tissue into specialized cells. Further investigations has been shown that only a small proportion of transplanted MSCs play such a role. On the other hand, MSCs produce a wide range of cytokines and chemokines which show strong local biological activity by means of paracrine action, and particularly via cell-derived extracellular vesicles [100-102]. These paracrine effects can facilitate stem cell homing and differentiation, but also create survival pathways for injured cell, as well as elicit anti-inflammatory and general reparative actions in damaged areas [103,104]. The question, what is more effective in the aspect of tissue regeneration, proper MSCs homing and differentiation in the defective area or their paracrine reparative action in this area, is open.

Potential clinical application

Neurological diseases

Number of potential clinical applications of placenta-derived and fetal membranes isolated cells is in constant growth, in particular because of their multilineage differentiation potential. Research aimed at intracerebral grafting of hAECs for the treatment of mouse model of Parkinson's disease showed that hAECs can synthesize and release catecholamine and neurotrophic factors such as nerve growth factor, neurotrophin-3and brain-derived neurotrophic factor [66,105,106].

Kong *et al.* [107] determined the survival and differentiation of human amniotic cells transplanted into the brain of MPTP induced Parkinson's disease (PD) mice. Results indicated that cells survived for at least 4 weeks after transplantation and promoted endogenous neurogenesis, though no morphological integration was observed.

Ischemic stroke occurs as a result of transient or permanent reduction in cerebral blood flow, resulting in cell death within few minutes. In order to treatment of this condition, Liu *et al.* [108] transplanted hAECs into ischemic rats, what resulted in significant ameliorate of behavioral dysfunction and also reduction of ischemic damage.

Heart diseases

Zhao *et al.* [75] demonstrated that hAMSCs present part of the characteristics of cardiomyocytes, which was confirmed by expression of multiple cardiac-related genes and proteins. Moreover, they indicated that unstimulated hAMSCs cultivated with heart explants can integrate into cardiac tissue and differentiate into cardiomyocyte-like cells. After transplantation freshly isolated hAMSCs into the myocardial infarcts in rat hearts, these cells survived in the scar tissue for at least 2 months and also differentiated into cardiomyocyte-like cells. The fact that hAMSCs can survive in xenotransplantation also suggest their low immunogenicity. These results give hope to use hAMSCs as a suitable source for the treatment of myocardial infarction in the future.

Lung fibrosis

Cargnoni *et al.* [109] investigated effects of fetal membrane-derived cells on a mouse model bleomycin-induced lung fibrosis. They isolated hAMSCs, hCMSCs and hAECs from fetal membranes and transplanted them as a mixture of mesenchymal and epithelial cells in bleomycin-treated mice, which represent a widely accepted model of lung interstitial fibrosis. They observed that intratracheal and intraperitoneal transplantation of cells results in a reduction in lung fibrosis process. These findings suggest that fetal membrane-derived cells may be useful for cell therapy of fibrotic diseases.

Liver disorders

There are evidence that hAECs are able to synthesis and secretion of albumin in a culture. Moreover, β-galactosidase-tagged hAECs transplanted into immunodeficient mice integrated into the liver parenchyma and could be detected until 7 day after transpalntation [67]. Albumin synthesis capacity, expression of liver lineage markers and low immunogenicity suggests their potential use in acute liver diseases.

CONCLUSIONS

Human fetal membranes are considered as an alternative and readily obtained tissue in the field of regenerative medicine. Unlimited availability of fetal membranes, which are routinely discarded postpartum, allow to isolate large number of stem cells from this tissues. Immunomodulatory properties and absence of ethical limitations make them extremely attractive and useful for stem cells based regenerative medicine and tissue engineering. Stromal and epithelial cells isolated from human fetal membranes display some characteristics of stem cells. They present great potential to differentiate into the all three germ layers cells: endoderm, mesoderm and ectoderm, which open a wide perspective of potential future clinical applications. Paracrine effects produced by MSCs also contribute to the repair processes in the damaged area. Nevertheless, further investigations are required to determine whether *in vitro* differentiation potential of these cells can be applied on a large scale in the treatment of many diseases.

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