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Amniotic membrane as a potent source of stem cells and a matrix for engineering heart tissue*

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ABSTRACT

Existing therapies for the treatment of chronic heart failure still have some limitations and there is a pressing need for the development of new therapeutic modalities. The amniotic membrane has been used for the treatment of various diseases, such as conjunctive defects; however, the mechanisms behind its repair functions are still unclear. Regenerative medicine is seeking newer alternatives and among them, biomaterials have emerged in recent years for developing and manipulating molecules, cells, tissues or organs grown in laboratories in order to replace human body parts. Many such materials have been used for this purpose, either synthetically or biologically, in order to provide new medical devices. This review provides a wider view of the regeneration potential of the use of amniotic membrane as a potential biomaterial to facilitate the implementation of new research in surgical procedures. Amniotic membrane appears to be an alternative source of stem cells as well as an excellent biomaterial for cell-based therapeutic applications in engineering heart tissue.

Keywords: Amniotic Membrane; Heart; Tissue; Engineering; Stem Cells

1. INTRODUCTION

The therapeutic options which are currently available for the treatment of some chronic cardiovascular diseases are still limited and palliative, highlighting the need for the development of new therapeutic modalities. Recent experimental studies have shown great potential, indicating the possibility of myocardial regeneration by the transplantation of biomaterials, and this has emerged as an alternative to existing therapies for heart injuries [1].

Amniotic membrane (AM) and amniotic fluid (AF) have attracted increasing attention in recent years as a possible reservoir for stem cells and also as promising sources of stem or progenitor cells that could be useful in clinical application in regenerative medicine [2,3].

Roubelakis *et al*. (2012) emphasised that AF and AM stem cells have the immunophenotypic characteristics of both adult mesenchymal stem cells and also embryonic stem cells. Consequently, these cells have been difficult to identify as they do not have markers and phenotypes. Roubelakis *et al*. (2012) proposed the use of a novel approach to identify them, based on transcriptomic, proteomic, or secretome analyses [4]. Dobreva *et al*. (2012)

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studied mice, and suggested the use of periostin as a biomarker of AM [5].

AM has been routinely used in several clinical studies for the treatment of burns on the skin, ulcers and, predominantly in ophthalmology, for the treatment of eyepiece surface disorders. Its use is based on its ability to improve the process of epithelisation, as well as reducing the inflammatory processes, angiogenesis and scarring alopecia [3,6,7].

Tissue-engineered technology provides the possibility of the enhanced recovery of injured tissues and organs. In general, this technology involves the selection of an appropriate substrate cultivation to sustain and promote the growth of a particular injured tissue. Three-dimensional substrates can be prepared, depending on the tissue in question. Several types of scaffolds are used: synthetic polymers that are not degradable; degradable synthetic polymers; collagen gel, without pores; non-human collagen gel, with human collagen tissue pores, and decellularised (cadaverous) [8].

In summary, none of the above-mentioned materials is entirely satisfactory in all respects, *i.e.* none are immunogenic, antitumoral, resistance and low cost. For this reason it is necessary to enhance a material for its use as a substrate for tissue engineering.

The implant composed of a collagen matrix seeded with cells, has demonstrated benefits in cardiac tissue, especially when compared to implants using isolated cells, without matrix. These benefits are extended when they are combined with other components of the extracellular matrix, such as growth factors. The amniotic fluid matrix has advantages because it has a surface area for cell cultivation with porosity, capillary growth, stability for mechanical support, biodegradability, and low immunogenicity [9-13].

Many materials have been used for this purpose, whether biological or synthetic, with the aim of producing new medical biomaterials. The purpose of this review is to provide a broader view of the basic biology of amniotic membrane and its potential for use in cardiac regeneration.

2. AMNIOTIC MEMBRANE

Human AM has been used as a biomaterial for surgical reconstruction for almost 100 years. There has been increasing interest in studying the biology of AM because it could eventually aid in the treatment of many ailments and improve the quality of human life. Thus, AM exhibits tremendous potential for therapeutic purposes due to its absorption, high biocompatibility, regenerative capacity and ease of implementation. It is easily accessible and is without ethical concerns [14].

The use of amniotic membrane originated at the beginning of the twentieth century, when, in 1910, Davis used it as surgical material for skin transplantation and subsequently for treating small skin defects in human patients [15]. In 1940, Roth described the transplantation of amniotic membrane for the first time in the repair of siblefaro and conjunctival defects [16]. In 1946, Sorby and Symons reported good results from the use of AM in the treatment of acute chemical burns, as well in other areas of medicine such as in the development of surgical dressings; the reconstruction of the oral cavity, bladder, tympanoplasty, arthroplasty and onfalocele; and preventing tissue adhesion in surgery of the head, abdomen, pelvis, vagina and larynx [6-12,16,17]. The aforementioned authors introduced the use of AM in ophthalmic surgery in experimental model and described the ability of AM to enhance wound healing, and epithelisation, noting an increase in the use of AM as a biomaterial in surgeries in recent decades. Similar data were submitted by other authors over the following years (**Table 1**) [15, 18-25].

3. BASIC STRUCTURE

The fetal membrane is composed of two main layers: the chorion, which is the outer layer of the placental mem-

Table 1. Amniotic membrane as a potential biomaterial for various organs and tissues. Studies in humans and animals.

Reference	Treatment of AM	Species	Organs/tissues
Davis (1910) [15]	Fresh	Human	Skin
Tseng *et al*. (1998) [18]	Cryopreserved	Human	Eyes
Azuara-Blanco *et al*. (1999) [19]	Cryopreserved	Human	Eyes
Chen *et al*. (2000) [20]	Cryopreserved	Human	Eyes
Mligiliche *et al*. (2002) [21]	Decellularised matrix	Rat	Peripheral nerve
Tamagawa *et al*. (2004) [22]	Fresh amniotic cells	Mouse	Hepatocyte
Lo *et al*. (2007) [23]	Fresh decellularised matrix	Rabbit	Skin
Zao *et al.* (2005) [24]	Amniotic Cells	Rat	Heart
Tsujhi *et al.* (2010) [25]	Amniotic Cells	Rat	Heart

brane which comes into contact with the maternal cells, and the AM, the innermost layer, which has intimate contact with the fetus, separated from it only by amniotic fluid. In the human species, AM appears 7 to 8 days after conception. Its thickness ranges from 0.02 mm to 0.5 mm and it has not direct blood supply or vascularisation [26].

Human AM, or amnion, is a translucent membrane composed of a layer of simple epithelium cells firmly adhered to the innermost layer, called the mesenchymal layer, which is composed of three layers: compact, spongy and fibroblastic. These layers contain large amounts of collagen (type IV and V) laminin and a thick basal layer as an array, without vascularity. Externally, the amnion is located in the chorion, comprising a connective tissue that presents fetal vessels (chorioallantoic) (**Figure 1**) [19].

Amniotic cells have numerous microvilli on their apical and ventral faces, extending their cellular processes to the basal membrane, and are known as podocytes. These cellular junctions are processes of adhesion to the basal membrane, of the hemidesmosome type with all their tonofilaments, and are located beneath the basal membrane, being material that is partially amorphous and microfibilar [27].

The basement membrane thickness in human tissue consists of type III, IV, VII collagen, elastin, fibronectin, perlecan and several other integrin complexes. The basement membrane is known for its healing, neovascularisation and anti-fibrosis properties [1,28].

Many pinocytic vesicles are found in the cytoplasm of cells from the AM, with, organelles in abundance, including nonlipid reticulum and Golgi apparatus. The cell cores have an irregular configuration and indents in the nuclear membrane, with large and homogeneous nucleoli, suggesting nucleolar activity. The ultra-structure of the amniotic membrane epithelium has specialised roles, such as the epithelium lining and as epithelial secretory with intense intercellular transport and trans-cellular activities. AM could be used to reduce inflammation in scars, enhancing the healing of wounds, and to serve as a substrate for proliferation and differentiation [29,30].

4. BIOLOGICAL PROPERTIES

Amniotic membrane has several biological properties; it reduces bacterial count and promotes the healing of infected wounds. This membrane is part of an important group of β-defensin antimicrobial peptides that are expressed in mucosal surfaces by epithelial cells and leukocytes, which are part of the innate immune system [31]. Its antibacterial property is attributed to its ability to adhere to the surface of wounds, to protect injuries and to reduce pain [32]. The innate immune system has evolved to eliminate microorganisms from entry in the tissues,

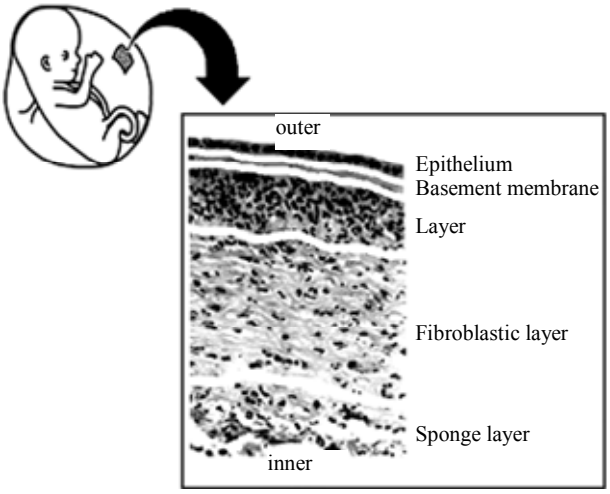

Figure 1. Histological scheme of human amniotic membrane showing its five layers.

creating antigens that are needed to produce an adaptive immune response. The absence of leucocytes in the amnion allows the use of the halo-transplant procedure, which does not induce rejection. The amniotic membrane stroma has several proteases that can inhibit the invasion of inflammatory cells, which might cause the rapid process of apoptosis [6].

Studies have shown the antimicrobial properties of anmiotic membrane and amniotic fluid in the healing process. The antimicrobial effect of amnion and chorion has been demonstrated against large numbers of bacterium, including *Streptococcus* Group A, *Staphylococci aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* [33].

Since 1960, there have been many reports demonstrating biological membranes as an implant material. Other studies have reported that biological membranes are organic in nature, inert, and composed almost exclusively of collagen, showing low antigenicity [34].

When amniotic membrane is preserved, it is regarded as an inert tissue with non-viable cells. The ability of this membrane to repair tissues occurs through the presence of growth factors and cytokines [33]. Other studies using AM have revealed the presence of various growth factors in the membrane epithelium, such as epidermal growth factor (EGF); transforming growth factor-β1 (TGF-β1); keratinocyte growth factor (KGF); fibroblastic growth factor (β-FGF); and hepatocyte growth factor (HGF), which act in facilitating cellular migration [35].

Bigbie *et al*. (1991) demonstrated the role of AM in the removal of granulation tissue and in promoting the decrease of exudation in wounds when they were treated with this membrane. According to Solomon *et al*. (2001), AM suppresses the expression of cellular epitopes as IL-1 α and IL-1 β cytocines. Another unique property of amniotic membrane is that it does not induce immune

rejection after transplantation because it does not express the histocompatibility antigens HLA-A, B, or DR, which makes it an excellent option for grafting [35-38].

Hao *et al*. (2000) reported that AM secretes some angiogenic factors, such as vascular endothelial growth factor (VEGF); interleukin-8 (IL-8); angiogenin; interferon-γ; interleukin-6 (IL-6); basic fibroblast growth factor (bFGF); epidermal growth factor (EGF) and platelet-derived growth factor (PDGF) [21,22]. It has also been demonstrated that some anti-angiogenic factors are released, such as IL-1 receptor antagonist, TIMP3 and TIMP4 [23]. Based on these data, AM can posses angiogenic and anti-angiogenic properties [38]. Perlecan induces high affinity binding of fibroblast growth factor (FGF)-2 to heparansulfate deficient cells or to soluble FGF receptors; it also possesses angiogenic properties [28].

Regarding the issue of low immunogenicity, clinical signs of acute infection and rejection were not observed when amniotic membrane was transplanted in volunteers [10]. The expression of MHC Class I antigens in AM is still controversial. Although it has been reported that HLA-A, B, C and DR was detected in amniotic epithelial cell culture, the detection of Class 1 antigens in nearly all cells from the amniotic membrane has been subsequently reported [10]. In addition, from the observation that amniotics cells disappeared without signs of reaction or rejection, it has been speculated that the short existence of these cells on the eyepiece surface may be related to the process of apoptosis [10,11].

There are reports in the literature of the several factors that are involved in the antifibrotic effect of amniotic membrane. Fibroblasts are responsible for the process of healing in wounds enabled by the transforming growth factor-β (TGF-β); the amniotic membrane has a regulatory mechanism of TGF-β and therefore reduces fibrosis [39,40].

Akle (1981) and Tamagawa (2004) reported that there was no evidence of tumorigenicity tests when they were isolated from amniotic membrane cells and transplanted in human volunteers to analyse immunogenicity [10,22].

Azuara-Blanco *et al*. (1999) and Dual *et al*. (2004) reported that amniotic membrane must be collected from a healthy donor who shows no signs of any type of infection, and through caesarean section, where its processing must be performed under sterile conditions [19,26].

Amniotic membrane contains two different types of cells: amnion epithelial cells (AECs) and amnion mesenchymal stromal cells (AMSCs). Human AECs (hAECs) are positive for the epithelial markers, cytokeratin 1, 2, 3, 4, 5, 6, 7, 8, 10, 13, 14, 15, 16 and 19 [40]. hAECs express embryonic stem cell markers, such as OCT-4, Nanog, Sox-2, Rex-1, SSEA3, SSEA4, TRA-1-60 and TRA-1-81, and other antigens such as ABCG2/BCRP (a member of the ATP-binding cassette super family), CD9, CD24, E-Cadherin, Integrin a6 and b and c-met (receptor growth factor of the hepatocyte) [41-45].

AMSCs express classical MSC markers (CD90, CD44, CD73, CD166, CD105 and CD29), as described for bone marrow stromal cells with the absence of hematopoietic markers (CD34 and CD45) and the concomitant lack of monocyte (CD114), macrophage (CD11) and fibroblast markers. Human AMSCs (hAMSCs) also express low levels of HLA-ABC but they do not express HLA-DR. AMSCs are also positive for the pluripotency markers Oct-4 and Nanog [43-49].

5. MECHANICAL PROPERTIES

Amniotic membrane has great mechanical strength, which makes it an attractive biomaterial to be used in surgery. This membrane is capable of supporting the load of the pressure of amniotic fluid and repetitive smaller loads, such as Braxton Hicks contractions during pregnancy.

In most cases, AM is able to withstand loads equal or close to physiological levels, soon after a transplant, in order to ensure its resistance. In addition, mechanical signals can be important mediators of differentiation for some progenitor cells; AM creates a suitable environment in the location of new transplanted tissue and it can increase the strength of the graft, with high levels of rigidity to resist the stress induced during tissue growth [23,41].

Other biomechanical properties of the amniotic fluid matrix, such as elasticity, rigidity and viscoelasticity, depend on the type of proteoglycans, collagen and elastin, which are important properties, the lack of which can promote intima layer hyperplasia and occlusion of the arteries [42].

6. *IN VITRO* CARDIOGENIC DIFFERENTIATION

The cardiogenic differentiation of human amniotic epithelial cells (hAECs) was first demonstrated by Miki *et al*. (2005). The RT-PCR of the 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 that were cultured in media supplemented with ascorbic acid for 14 days. Differentiated cardiomyocytes have also expressed α-actinin, which has been demonstrated by immunocytochemistry [50].

Ilancheran *et al*. (2007) showed that freshly isolated native hAECs, and those grown under standard conditions, also expressed the mRNAs of genes that are important for the specification of the cardiomyoctyic lineage, such as GATA4, and function, including ANP,

MYL7, CACNA1C, and KCND3. However, ultrastructural analysis analysis has revealed that features consistent with relatively mature cardiomyocytes, such as myofilaments, myofibrils, H bands and T tubules, were present only in differentiated cells [42].

The myogenic differentiation of hAMSCs has been determined by RT-PCR since Portmann-Lanz *et al*. (2006) demonstrated the mRNA expression of myogenic transcription factors such as myoD and myogenin, and the protein expression of desmin in hAMSCs after induction with differentiation media [51].

Fetal maternal stem cell transfer appears to be a critical mechanism in the maternal response to cardiac injury. Furthermore, caudal-related homeobox2 (Cdx2) cells have been identified as a novel cell type for potential use in cardiovascular regenerative therapy [52].

The use of amniotic membrane as a source for cardiac regeneration offers excellent advantages in that it is in plentiful supply and it can be applied immediately, without the need for any cell isolation, which makes it economical. In addition, amniotic membrane has good preservability and is immunologically tolerated in allogeneic conditions; it has shown cardiogenic differentiation *in vitro* and *in vivo*.

7. PREPARATION AND PRESERVATION

Kim and Tseng, in 1995 and 1998, proposed a method for the preparation and preservation of amniotic membrane, consisting of collecting the placenta through caesarean section in an environment free from contamination, removing clots through rinsing with saline solution, separating the chorion amnion manually, and inserting it into nitrocelullose with the epithelial surface upwards. In most studies, the preservation of the membrane follows the protocol in which the membrane is prepared with antibiotics and antifungals and kept in medium containing antibiotics and glycerol at −80˚C [7].

There are reports in the literature about other methods of preservation and storage of amniotic membrane, including freeze-drying, dry air, treatment with glutaraldehyde, dispase, gamma irradiation [53]. Dual *et al*. (2004) and Mejia *et al*. (2002) reported that both preserved and non-preserved amniotic membrane can be stored. Dimethyl sulfoxide (DMSO) has been used as an alternative to the preservation of amniotic membrane by replacing the rinsing with saline and antibiotics; Azuara-Blanco *et al*. (1999) used DMSO to 4, 8 and 10, while Kubo *et al*. (2001) used the same product to 0.5, 1.0 and 1.5 M for the washing membrane. After such treatment, it may be stored in 1.5 M DMSO to −80˚C for several months [11,19,26,54].

Trehalose is a reductor disaccharide, which is present in high concentrations in various organisms and is capable of surviving almost complete dehydration [55]. Nakamura *et al*. (2008) used it to stabilise and preserve cell membrane proteins. Some studies have demonstrated the combination of freeze-drying and gamma irradiation of AM as an efficient sterilisation technique. However, the use of a chemical family of organic peroxides, peracetic acid, is usually used as a sterilising agent against many viruses, bacterium and spores, due to its high oxidation potential and non-toxic waste generation. In a study by Wilshaw *et al.* (2006), the use of peracetic acid to sterilise human skin was effective, as it preserved the extracellular matrix components, such as type IV collagen, laminin, fibronectin, elastin and glycosaminoglycans. Furthermore, there was no significant reduction in voltage, resistance and elasticity after the treatment of amniotic membrane with peracetic acid [56].

Souza *et al.* (2004) reported the contamination of amniotic membrane after caesarean section delivery. These authors also described the contamination of amniotic fluid in 13 of 23 patients with intact membranes and suggested aseptic care before handling the membrane. Dual *et al*. (2004) argue that there is a risk of infection, and that disinfecting procedures must be performed, not only in preparation and storage, but also during clinical use [25,57].

The use of amniotic membrane associated with tissue bioengineering is a powerful tool in the repair, protection and reconstruction of various organs and tissues.

8. ENGINEERING HEART TISSUE

Cargnoni *et al*. (2009) used a fragment of AM that was applied to the left ventricle after coronary artery ligation by ischaemia in rats. Seven days after the transplant, the rats treated with amniotic membrane showed a greater preservation of cardiac dimensions and cardiac contractile element function, and they had improved in terms of the largest left ventricle ejection fraction shortening and thickening of the wall. It has been suggested that the use of amniotic membrane can be the vehicle for the supply of cells that are able to produce soluble factors and cardioprotectors, and this reinforces the notion that this tissue is a source of cells with clinical potential that has not been fully revealed [58].

A study was performed using transplanted amniotic membrane cells (cells that are derived from the mesodermal lineage). When these cells were transplanted into rat myocardium in myocardial infarction, the analysis of heart function demonstrated that 2 and 6 weeks after the implantation there was no attenuation or improvement of dilation of the left ventricular dilatation and maintenance of cardiac dysfunction, when compared to the control group [59].

Zhao *et al*. (2005) used isolated cells from amniotic membrane that were analysed by the PCR technique for the detection of specific cardiac genes and subsequently

transplanted them in heart infarction in murine model. They described that the cells from the amniotic membrane survived in scar tissue for at least two months afterwards and these cell were differentiated from cells with characteristics of cardiomyocytes [24].

Lionetti *et al*. (2010) reported that transplantation of human amnion-derived mesenchymal stem cells with hyaluron in mixed esters of butyric and retinoic acid conditions in rat injured myocardium, differentiated into cardiomyocyte phenotype and increased the capillary density of the infarcted myocardium [60].

Tsujhi *et al*. (2010) studied stem cells derived from rat heart that were transplanted in AM and were able to be transdiferenciated in cardiomyocytes. They demonstrated immunological tolerance *in vivo* for four weeks after the transplant. Those experiments were performed without the use of immunosuppressive agents [29].

Studies suggest that the cells of AM have some common characteristics with cardiomyocytes, and there is the possibility of them being suitable for cellular cardiomyoplasty, although their characteristics and potential are not yet completely understood [24,49]. In the potential alternative therapeutic applications in engineering heart tissue, there should be kinds of disadvantages to be taken into consideration: allogeneic reactions and the virus contamination of AM, as Cytomegalovirus [61]. Those conditions can be avoided with the use of immunosuppressant and gamma radiation into End-products: matrix or cells, respectively.

9. CONCLUSION

AM is an alternative source of stem cells, which is particularly interesting due to its ability to differentiate, low immunogenicity, low carcinogenicity, as well as being without any ethical concerns. AM has great potential for therapeutic applications in regenerating heart tissue and is a potential source of mesenchymal stem cells, as well as the source of biocompatible matrix.

REFERENCES

[1] Chachques, J.C. (2011) Development of bioartificial myocardium using stem cells and nanobiotechnology templates. *Cardiology Research and Practice*, **2011**, Article ID: 806795. http://dx.doi.org/10.4061/2011/806795

[2] Toda, A., Okabe, M., Yoshida, T. and Nikaido, T. (2007) The potential of amniotic membrane/amnion-derived cells for regeneration of various tissues. *Journal of Pharmacology Sciences*, **105**, 215-218.

[3] Insausti, C.L., Blanquer, M., Bleda, P., Iniesta, P., Majado, M.J., Castellanos, G. and Moraleda, J.M. (2010) The amniotic membrane as a source of stem cells. *Histology and Histopathology*, **25**, 91-98.

[4] Roubelakis, M.G., Trohatou, O. and Anagnou, N.P. (2012) Amniotic fluid and aminiotic membrane stem cells: Marker discovery. *Stem Cells International*, **2012**, Article ID: 107836. http://dx.doi.org/10.1155/2012/107836

[5] Dobreva, M.P., Lhoest, L., Pereira, P.N., Umans, L., Camus, A., Lopes, S.M.C.S. and Zwijsen, A. (2012) Periostin as a biomarker of the amniotic membrane. *Stem Cells International*, **2012**, Article ID: 987185. http://dx.doi.org/10.1155/2012/987185

[6] Trelford, J.D. and Trelford-Sauder, M. (1979) The amnion in surgery, past and present. *American Journal of Obstetrics & Gynecology*, **134**, 833-845.

[7] Kim, J.C. and Tseng, S.C. (1995) Transplantation of preserved human amniotic membrane for surface reconstruction in severely damaged rabbit corneas. *Cornea*, **14**, 473-484. http://dx.doi.org/10.1097/00003226-199509000-00006

[8] Lindenmair, A., Wolbank, S., Stadler, G., Meinl, A., Peterbauer-Scherb, A., Eibl, J., Polin, H., Gabriel, C., Griensven, M. and Redl, H. (2010) Osteogenic differentiation of intact human amniotic membrane. *Biomaterials*, **31**, 8659-8665. http://dx.doi.org/10.1016/j.biomaterials.2010.07.090

[9] Zimmermann, W.H. and Eschenhagen, T. (2003) Cardiac tissue engineering for replacement therapy. *Heart Failure Reviews*, **8**, 259-269. http://dx.doi.org/10.1023/A:1024725818835

[10] Akle, C.A., Adinolfi, M., Welsh, K.I., Leibowitz, S. and McColl, I. (1981) Immunogenicity of human amniotic epithelial cells after transplantation into volunteers. *Lancet*, **318**, 1003-1005. http://dx.doi.org/10.1016/S0140-6736(81)91212-5

[11] Kubo, M., Sonoda, Y., Muramatsu, R. and Usui, M. (2001) Immunogenicity of human amniotic membrane in experimental xenotransplantation. *Investigative Ophthalmology & Visual Science*, **42**, 1539-1546.

[12] Niknejad, H., Peirovi, H., Jorjani, M., Ahmadiani, A., Ghanavi, J. and Seifalian, A.M. (2008) Properties of the amniotic membrane for potential use in tissue engineering. *European Cells and Materials Journal*, **15**, 88-99.

[13] Zhang, D.G., Jiang, M.Y. and Miao, D.S. (2011) Transplanted human amniotic membrane-derived mesenchymal stem cells ameliorate carbon tetrachloride-induced liver cirrhosis in mouse. *PLoS One*, **6**, Article ID: e16789.

[14] Lindenmair, A., Hatlapatka, T., Kollwig, G., Hennerbichler, S., Gabriel, C., Wolbank, S., Redl, H. and Kasper, C. (2012) Mesenchymal stem or stromal cells from amnion and umbilical cord tissue and their potential for clinical applications. *Cells*, **1**, 1061-1088. http://dx.doi.org/10.3390/cells1041061

[15] Davis, J.W. (1910) Skin transplantation with a review of 550 cases at the Johns Hopkins Hospital. *Johns Hopkins Medical Journal*, **15**, 307.

[16] Roth, A.D. (1940) Plastic repair of conjunctival defects with fetal membrane. *Archives of Ophthalmology*, **23**, 522-525. http://dx.doi.org/10.1001/archopht.1940.00860130586006

[17] Sorsby, A. and Symons, H.M. (1946) Amniotic membrane grafts in caustic burns of the eye (Burns of the

second degree). *British Journal of Ophthalmology*, **30**, 337-345. http://dx.doi.org/10.1136/bjo.30.6.337

[18] Tseng, S.C., Prabhasawat, P., Barton, K., Gray, T. and Meller, D. (1998) Amniotic membrane transplantation with or without limbal allografts for corneal surface reconstruction in patients with limbal stem cell deficiency. *Archives of Ophthalmology*, **116**, 431-441. http://dx.doi.org/10.1001/archopht.116.4.431

[19] Azuara-Blanco, A., Pillai, C.T. and Dua, H.S. (1999) Amniotic membrane transplantation for ocular surface reconstruction. *British Journal of Ophthalmology*, **83**, 399-402. http://dx.doi.org/10.1136/bjo.83.4.399

[20] Chen, H.J., Pires, R.T. and Tseng, S.C. (2000) Amniotic membrane transplantation for severe neurotrophic corneal ulcers. *British Journal of Ophthalmology*, **84**, 826-833. http://dx.doi.org/10.1136/bjo.84.8.826

[21] Mligiliche, N., Endo, K., Okamoto, K., Fujimoto, E. and Ide, C. (2002) Extracellular matrix of human amnion manufactured into tubes as conduits for peripheral nerve regeneration. *Journal ofBiomedical Material Research*, **63**, 591-600. http://dx.doi.org/10.1002/jbm.10349

[22] Tamagawa, T., Ishiwata, I. and Saito, S. (2004) Establishment and characterization of a pluripotent stem cell line derived from human amniotic membranes and initiation of germ layers in vitro. *Human Cell*, **17**, 125-130. http://dx.doi.org/10.1111/j.1749-0774.2004.tb00028.x

[23] Lo, V. and Pope, E. (2009) Amniotic membrane use in dermatology. *International Journal of Dermatology*, **48**, 935-940. http://dx.doi.org/10.1111/j.1365-4632.2009.04173.x

[24] Zhao, P., Ise, H., Hongo, M., Ota, M., Konishi, I. and Nikaido, T. (2005) Human amniotic mesenchymal cells have some characteristics of cardiomyocytes. *Transplantation*, **79**, 528-535. http://dx.doi.org/10.1097/01.TP.0000149503.92433.39

[25] Tsuji, H., Miyoshi, S., Ikegami, Y., Hida, N., Asada, H., Togashi, I., Suzuki, J., Satake, M., Nakamizo, H., Tanaka, M., Mori, T., Segawa, K., Nishiyama, N., Inoue, J., Makino, H., Miyado, K., Ogawa, S., Yoshimura, Y. and Umezawa, A. (2010) Xenografted human amniotic membrane-derived mesenchymal stem cells are immunologically tolerated and transdifferentiated into cardiomyocytes. *Circulation Research*, **106**, 1613-1623. http://dx.doi.org/10.1161/CIRCRESAHA.109.205260

[26] Dual, H.S. and Azuara-Blanco, A. (1999) Amniotic membrane transplantation. *British Journal of Ophthalmology*, **83**, 748-752. http://dx.doi.org/10.1136/bjo.83.6.748

[27] Wynn, R.M. and Corbett, J.R. (1969) Ultrastructure of the canine placenta and amnion. *American Journal of Obstetrics & Gynecology*, **103**, 878-887.

[28] Segev, A., Nili, N. and Strauss, B.H. (2004) The role of perlecan in arterial injury and angiogenesis. *Cardiovascular Research*, **63**, 603-610. http://dx.doi.org/10.1016/j.cardiores.2004.03.028

[29] Sachs, B.P. and Stern, C.M. (1979) Activity and characterization of a low molecular fraction present in human amniotic fluid with broad spectrum antibacterial activity. *British Journal of Obstetrics & Gynecology*, **86**, 81-86. http://dx.doi.org/10.1111/j.1471-0528.1979.tb10572.x

[30] Kim, J.C. and Tseng, S.C. (1995) The effects on inhibition of corneal neovascularization after human amniotic membrane transplantation in severely damaged rabbit corneas. *Korean Journal of Ophthalmology*, **9**, 32-46.

[31] Krisanaprakornkit, S., Weinberg, A., Perez, C.N. and Dale, B.A. (1998) Expression of the peptide antibiotic human beta-defensin 1 in cultured gingival epithelial cells and gingival tissue. *Infection and Immunity*, **66**, 4222-4228.

[32] Talmi, Y.P., Sigler, L., Inge, E., Finkelstein, Y. and Zohar, Y. (1991) Antibacterial properties of human amniotic membranes. *Placenta*, **12**, 285-288. http://dx.doi.org/10.1016/0143-4004(91)90010-D

[33] Dua, H.S., Gomes, J.A., King, A.J. and Maharajan, V.S. (2004) The amniotic membrane in ophthalmology. *Survey of Ophthalmology*, **49**, 51-77. http://dx.doi.org/10.1016/j.survophthal.2003.10.004

[34] Cen, L., Liu, W., Cui, W., Zhang, W. and Cao, Y. (2008) Collagen tissue engineering: Development of novel biomaterials and applications. *Pediatric Research*, **63**, 492-496. http://dx.doi.org/10.1203/PDR.0b013e31816c5bc3

[35] Koizumi, N., Fullwood, N.J., Bairaktaris, G., Inatomi, T., Kinoshita, S. and Quantock, A.J. (2000) Cultivation of corneal epithelial cells on intact and denuded human amniotic membrane. *Investigative Ophthalmology & Visual Science*, **41**, 2506-2513.

[36] Hopper, R.A., Woodhouse, K. and Semple, J.L. (2003) Acellularization of human placenta with preservation of the basement membrane: A potential matrix for tissue engineering. *Annals of Plastic Surgery*, **51**, 598-602. http://dx.doi.org/10.1097/01.sap.0000095658.46675.76

[37] Shortt, A.J., Secker, G.A., Lomas, R.J., Wilshaw, S.P., Kearney, J.N., Tuft, S.J. and Daniels, J.T. (2008) The effect of amniotic membrane preparation method on its ability to serve as a substrate for the *ex-vivo* expansion of limbal epithelial cells. *Biomaterials*, **30**, 1056-1065. http://dx.doi.org/10.1016/j.biomaterials.2008.10.048

[38] Bigbie, R.B., Schumacher, J., Swaim, S.F., Purohit, R.C. and Wright, J.C. (1991) Effects of amnion and live yeast cell derivative on second-intention healing in horses. *American Journal of Veterinary Research*, **52**, 1376-1382.

[39] Solomon, A., Rosenblatt, M., Monroy, D., Ji, Z., Pflugfelder, S.C. and Tseng, S.C. (2001) Suppression of interleukin 1alpha and interleukin 1beta in human limbal epithelial cells cultured on the amniotic membrane stromal matrix. *British Journal of Ophthalmology*, **85**, 444-449. http://dx.doi.org/10.1136/bjo.85.4.444

[40] Hao, Y., Ma, D.H., Hwang, D.G., Kim, W.S. and Zhang, F. (2000) Identification of antiangiogenic and antiinflammatory proteins in human amniotic membrane. *Cornea*, **19**, 348-352. http://dx.doi.org/10.1097/00003226-200005000-00018

[41] Díaz-Prado, S., Muiños-López, E., Hermida-Gomez, T., Rendal-Vazquez, M.E., Fuentes-Boquete, I., de Toro, F.J. and Blanco, F.J. (2011) Isolation and characterization of mesenchymal stem cells from human amniotic membrane. *Tissue Engineering Part C*: *Methods*, **17**, 49-59. http://dx.doi.org/10.1089/ten.tec.2010.0136

[42] Ilancheran, S., Moodley, Y. and Manuelpillai, U. (2009)

Human fetal membranes: A source of stem cells for tissue regeneration and repair? *Placenta*, **30**, 2-10. http://dx.doi.org/10.1016/j.placenta.2008.09.009

[43] Parolini, O., Soncini, M., Evangelista, M. and Schmidt, D. (2009) Amniotic membrane and amniotic fluid-derived cells: Potential tools for regenerative medicine? *Regenerative Medicine*, **4**, 275-291. http://dx.doi.org/10.2217/17460751.4.2.275

[44] Niknejad, H., Peirovi, H., Jorjani, M., Ahmadiani, A., Ghanavi, J. and Seifalian, A.M. (2009) Properties of the amniotic membrane for potential use in tissue engineering. *European Cells & Materials Journal*, **15**, 88-99.

[45] Insausti, C.L., Blanquer, M., Bleda, P., Iniesta, P., Majado, M.J., Castellanos, G. and Moraleda, J.M. (2010) The amniotic membrane as a source of stem cells. *Histology and Histopathology*, **25**, 91-98.

[46] Kobayashi, M., Yakuwa, T., Sasaki, K., Sato, K., Kikuchi, A., Kamo, I., Yokoyama, Y. and Sakuragawa, N. (2008) Multilineage potential of side population cells from human amnion mesenchymal layer. *Cell Transplantion*, **17**, 291-301. http://dx.doi.org/10.3727/096368908784153904

[47] Mihu, C.M., RusCiuča, D., Soritău, O., Susman, S. and Mihu, D. (2009) Isolation and characterization of mesenchymal stem cells from the amniotic membrane. *Romanian Journal of Morphology and Embryology*, **50**, 73-77.

[48] Miki, T. and Strom, S.C. (2006) Amnion-derived pluripotent/multipotent stem cells. *Stem Cell Reviews*, **2**, 133-142. http://dx.doi.org/10.1007/s12015-006-0020-0

[49] Paracchini, V., Carbone, A., Colombo, F., Castellani, S., Mazzucchelli, S., Di Gioia, S., Degiorgio, D., Seia, M., Porretti, L., Colombo, C. and Conese, M. (2012) Amniotic mesenchymal stem cells: A new source for hepatocyte-like cells and induction of CFTR expression by coculture with cystic fibrosis airway epithelial cells. *Journal of Biomedicine and Biotechnology*, **2012**, Article ID: 575471. http://dx.doi.org/10.1155/2012/575471

[50] Miki, T., Lehmann, T., Cai, H., Stolz, D.B. and Strom, S.C. (2005) Stem cell characteristics of amniotic epithelial cells. *Stem Cells*, **23**, 1549-1559. http://dx.doi.org/10.1634/stemcells.2004-0357

[51] Portmann-Lanz, C.B., Schoeberlein, A., Huber, A., Sager, R., Malek, A., Holzgreve, W. and Surbek, D.V. (2006) Placental mesenchymal stem cells as potential autologous graft for pre- and perinatal neuroregeneration. *American Journal of Obstetrics & Gynecology*, **194**, 664-673. http://dx.doi.org/10.1016/j.ajog.2006.01.101

[52] Kara, R.J., Bolli, P., Karakikes, I., Matsunaga, I., Tripodi, J., Tanweer, O., Altman, P., Shachter, N.S., Nakano, A., Najfeld, V. and Chaudhry, H.W. (2012) Fetal cells traffic to injured maternal myocardium and undergo cardiac differentiation. *Circulation Research*, **110**, 82-93. http://dx.doi.org/10.1161/CIRCRESAHA.111.249037

[53] Choi, T.H. and Tseng, S.C. (2001) *In Vivo* and *in Vitro* demonstration of epithelial cell-induced myofibroblast differentiation of keratocytes and an inhibitory effect by amniotic membrane. *Cornea*, **20**, 197-204. http://dx.doi.org/10.1097/00003226-200103000-00019

[54] Mejia, L.F., Santamaria, J.P. and Acosta, C. (2002) Symptomatic management of postoperative bullous keratopathy with nonpreserved human amniotic membrane. *Cornea*, **21**, 342-345. http://dx.doi.org/10.1097/00003226-200205000-00002

[55] Nakamura, T., Sekiyama, E., Takaoka, M., Bentley, A.J., Yokoi, N., Fullwood, N.J. and Kinoshita, S. (2008) The use of trehalose-treated freeze-dried amniotic membrane for ocular surface reconstruction. *Biomaterials*, **29**, 3729-3737. http://dx.doi.org/10.1016/j.biomaterials.2008.05.023

[56] Wilshaw, S.P., Kearney, J.N., Fisher, J. and Ingham, E. (2006) Production of an acellular amniotic membrane matrix for use in tissue engineering. *Tissue Engineering*, **12**, 2117-2129. http://dx.doi.org/10.1089/ten.2006.12.2117

[57] Souza, C.E.B., Engel, D.P., Branco, B.C., Hofling-Lima, A.L., Freitas, D., Gomes, J.A.P. and Souza, L.B. (2004) Evaluation of microbiological contamination of amniotic membrane and amniotic fluid. *Arquivos Brasileiros de Oftalmologia*, **67**, 709-712. http://dx.doi.org/10.1590/S0004-27492004000500003

[58] Cargnoni, A., Di Marcello, M., Campagnol, M., Nassuato, C., Albertini, A. and Parolini, O. (2009) Amniotic membrane patching promotes ischemic rat heart repair. *Cell Transplantation*, **18**, 1147-1159. http://dx.doi.org/10.3727/096368909X12483162196764

[59] Fujimoto, K.L., Miki, T., Liu, L.J., Hashizume, R., Strom, S.C., Wagner, W.R., Keller, B.B. and Tobita, K. (2009) Naive rat amnion-derived cell transplantation improved left ventricular function and reduced myocardial scar of postinfarcted heart. *Cell Transplantation*, **18**, 477-486. http://dx.doi.org/10.3727/096368909788809785

[60] Lionetti, V., Cantoni, S., Cavallini, C., Bianchi, F., Valente, S., Frascari, I., Olivi, E., Aquaro, G.D., Bonavita, F., Scarlata, I., Maioli, M., Vaccari, V., Tassinari, R., Bartoli, A., Recchia, F.A., Pasquinelli, G. and Ventura, C. (2010) Hyaluronan mixed esters of butyric and retinoic acid affording myocardial survival and repair without stem cell transplantation. *The Journal of Biological Chemistry*, **285**, 9949-9961. http://dx.doi.org/10.1074/jbc.M109.087254

[61] Matsunaga, S., Uchide, N., Shono, M., Ohyama, K., Takeichi, M. and Toyoda, H. (2013) Differences in permissive cytomegalovirus infection between primary cultured human fetal membrane chorion and amnion cells. *Biological and Pharmaceutical Bulletin*, **36**, 1715-1721. http://dx.doi.org/10.1248/bpb.b13-00200

