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Could human amniotic membrane be a source for acupoint thread embedding therapy?

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Abstract

Background: Peripheral neuropathy usually leads to a major cause of motor disability, but the functional restoration after treatment continues to show modest results. Acupoint thread-embedding therapy is a subtype of acupuncture treatment in which biodegradable threads are inserted into skin, subcutaneous tissue or muscles at specific points for long stimulation. Different biodegradable materials have been developed and widely used. Human amniotic membrane is rich in collagen, extracellular matrix proteins and growth factors. The avascular, low immunogenic, anti-inflammatory, antibacterial, anti-fibrotic and non-tumorigenic properties of amniotic membrane make it valuable in medical applications and its use has no ethical problems. Elasticity, stiffness and other biomechanical properties also make it possible to use the amniotic membrane for various medical purposes. AM is almost always considered as discarded substance, it satisfies most of the criteria of an ideal biological tissue and shows almost zero rejection phenomenon.
Conclusions: The human amniotic membrane, the cellular compounds and extracellular matrix have a lot of benefic proprieties that are or could be used in treatment of many human diseases. Its biological and biomechanical properties are promising in the manufacture and use of filaments in acupoint thread embedding therapy.
Key words: peripheral neuropathy, acupoint thread embedding therapy, amniotic membrane.

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Introduction

Peripheral neuropathy is a damage or dysfunction of one or more peripheral nerves, which usually leads to numbness, tingling, muscle weakness, and pain in the affected area [1]. Globally, it is estimated that 2-3% of the population suffers from peripheral neuropathy, and the prevalence increases with age [2]. A study estimated that the prevalence of peripheral neuropathy in family medicine is 8% in people from 55 years old [3]. Peripheral nerve trauma remains a major cause of motor disability, at the same time functional restoration after treatment continues to show modest results [4].

In 1960s, in China appeared a treatment method by implanting absorbable materials (e.g. catgut) substituting for filiform needles into the acupoints, which realized long-time needle retaining and also avoided the danger of filiform needle retaining. This method was then termed acupoint thread-embedding therapy (ATET) [5]. ATET is an invasive treatment which can prolong point stimulation, reduces the frequencies of pain and psychological fear of patients [4, 6]. Different biodegradable materials have been developed and widely used. They are divided into natural (e.g., catgut) and synthetic types (e.g., polyglycolic acid, polylactic acid etc.) according to material sources. Both have advantages and disadvantages [6]. The ideal embedding materials are required to be safe, non-toxic, biocompatible, and to have excellent swelling and biodegradation behaviors. ATET can be a promising treatment method of peripheral nerve disorders [4].

The amniotic membrane (AM) is a biological material of the human placenta, constituting the inner wall of the fetal membranes [7]. It surrounds the embryo/fetus and delimits the amniotic cavity, which is filled by amniotic fluid [8]. Fetal membranes are composed of two layers: an outer layer (chorion), which contacts maternal cells and an inner layer (amniotic membrane) [9].

AM is a gift of nature which not only protects the fetus inside the womb but also has several medicinal proper-

ties. It serves as a natural barricade to protect the fetus from bacterial infection and trauma [10]. The fetal membranes facilitate the exchange of gas, nutrients, and waste, serving as a barrier to protect fetus from the maternal immune system and synthesizing certain hormones and enzymes that are critical during pregnancy and parturition [11-14]. AM is not just a simple avascular structure; it has multiple metabolic functions, such as the transport of water and soluble materials and the production of bioactive factors, including vasoactive peptides, growth factors and cytokines [15, 16].

Although AM is almost always considered as discarded substance, it satisfies most of the criteria of an ideal biological dressing and shows almost zero rejection phenomenon [17]. It is one of the thickest membranes in the human body and can withstand current cryopreservation techniques [15, 18]. The translucent, avascular, low immunogenic, anti-inflammatory, anti-scarring, and wound healing properties of AM allow this material function beyond its role *in vivo* and assume a wide range of applications in regenerative medicine [19, 20].

The earliest known clinical applications of amnion go back to the beginning of the previous century. Until the beginning of the seventies only a few reports can be found. The use of amnion has become firmly established in addition to other new methods [21].

The literature search was performed using the search terms "peripheral neuropathy", "acupoint thread embedding therapy", "amniotic membrane" and were selected from databases, such as PubMed, Hinari, Springer, Elsevier and Science Direct. The material was selected based on the studies published until 13/01/ 2021, which aimed to elucidate the structure and properties of human amniotic membrane. After processing the data obtained from databases according to the search criteria, we found 107 articles related to human amniotic membrane properties and structure. The articles used were written in English and Romanian. The final bibliography included 97 relevant sources that were considered representative materials on this topic and sufficient to formulate the main ideas of this text. The information systematized the main aspects of human amniotic membrane structure and properties. The articles which do not correspond to this article goal were excluded.

Amniotic membrane structure

AM thickness varies from 0.02 mm to 0.05 mm and consists of three main histological layers: the epithelial layer, the thick basement membrane and the avascular mesenchymal tissue or stroma [18]. The AM contains no blood vessels or nerves; instead, the nutrients it requires are supplied directly by diffusion out of the amniotic fluid and/or from the underlining decidua. Two cell types, extracellular matrix proteins, and growth factors are placed in the mentioned layers [19, 22].

Epithelium is a monolayer of metabolically active cuboidal cells with microvilli present on its apical surface which are in direct contact with amniotic fluid [18, 20]. These cells have a large irregular nucleus with a large homogeneous nucleolus and many intracytoplasmic organelles and pinocytic vesicles [23]. The first epithelial layer is composed of collagen I, II, and V and expresses some of the crucial epidermal markers, such as glycoprotein CA125 and oxytocin receptors and are also positive for antigen CD44 and desmin [24, 25]. Erythropoietin and its receptors are expressed in human amniotic epithelial cells. Erythropoietin, whose functions are still unknown in the AM, stimulates the differentiation, proliferation and survival of erythroid precursors and its production is regulated by the concentration of oxygen in the blood [22, 26].

Ogawa et al. (2003) have reported that erythropoietin production in human amniotic epithelial cells is stimulated by progesterone but is not stimulated by hypoxia or 17β-estradiol [26].

The basement membrane is made up by reticular fibers. It is made up of type IV, V and VII collagen in addition to fibronectin and laminin. Although thin, it is one of the thickest basement membranes found in the human body and can withstand cryopreservation [18, 27]. The basement membrane contains large amounts of proteoglycans that are rich in heparan sulphate and that serve as a permeable barrier to amniotic macromolecules and several molecules with a structural function enabling the maintenance of membrane integrity. These molecules are actin, α-actinin, spectrin, ezrin, several cytokeratins, vimentin, desmoplakin and laminin [28-30].

The stroma of AM can be subdivided further into a compact layer, a fibroblast layer, and an outer spongy layer [18]. The collagens of the compact layer are secreted by mesenchymal cells situated in the fibroblast layer. Interstitial collagens (types I and III) predominate and form parallel bundles that maintain the mechanical integrity of AM. Collagens type V and VI form filamentous connections between interstitial collagens and the epithelial basement membrane. The spongy layer of the stromal matrix sits adjacent to the chorionic membrane. Its abundant content of proteoglycans and glycoproteins produces a spongy appearance in histologic preparations, and it contains a nonfibrillar meshwork of mostly type III collagen [31]. Closely connected to the chorionic membrane, the spongy layer consists of wavy bundles of reticulum bathed in mucin; hence, AM is easily separated from the chorion by means of blunt dissection [19].

Amnion-derived cells

Cells of AM have pluripotent properties and, for this reason, are an attractive source for transplantation [24]. Pluripotent stem cells are self-renewing cells, capable for differentiating into all 3 germ layers of the developing embryo: ectoderm, mesoderm, and endoderm. The amniotic membrane includes amniotic mesenchymal cells (AMCs) and amniotic epithelial cells (AECs) which are responsible for the production of extracellular matrix (ECM), different cytokines and growth factors [19]. These cells have several properties which make them as an appropriate cell source for stem cell therapy. AMCs exhibited plastic adherence and fibroblastic morphology, while AECs displayed a cobblestone epithelial phenotype [32, 33]. One of the most abundant proteins found in AM derived cells is laminin, which

plays a key role in differentiation, cell shape and migration, and tissue regeneration [24, 34]. Several studies have documented that amniotic cells express various surface markers associated with embryonic stem cells, e.g. stage-specific embryonic antigen 3 and 4 (SSEA-3 and –4), TRA-1-60 and TRA-1-81 [19]. Epithelial and mesenchymal amniotic cells also express various stem cell markers, such as octamer-binding transcription factor 4 (OCT-4), hepatocyte nuclear factor 3β (HNF-3β), nanog and nestin [32, 35, 36].

The mesenchimal stem cells (MSCs) derived from amniotic membrane have been reported as a better new prospective field of regenerative medicine compared with other MSCs sources, because of the easiness of their acquisition, reduced donor damage, multipotency, low immune response, acceptable ethical issue [24, 37]. The AMCs are positively expressed for the mesenchymal specific markers including CD44, CD73, CD29, CD105 and CD90 and do not express the hematopoietic markers and human leukocyte antigen including CD34, CD45, CD11b, CD19, HLA-A, HLA-B and DR antigens [38]. AMCs also secrete some anti-inflammatory cytokines, such as PGE2, IDO, HGF and TGF-b [39]. AMCs promote angiogenesis through secretion of some angiogenic factors, such as angiogenin, vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) [40]. RT-PCR analysis showed that AMSCs expressed genes, such as Oct-3/4, zinc finger protein 42 (zfp42 or Rex-1), stem cell factor protein (SCF), neural cell adhesion molecule (NCAM), nestin (NES), bone morphogenetic protein 4 (BMP-4), GATA binding protein 4 (GATA-4), and hepatocyte nuclear factor 4α (HNF-4α) even in high passages [32, 38]. AMCs shared similar phenotypic characteristics with the ones derived from adult sources. AMCs exhibited a higher proliferation rate compared to MSCs derived from adult sources and a multilineage differentiation potential into cells derived from the three germ layers [32, 33, 37].

AECs have some properties that make them a precious candidate to be considered as a source of pluripotent stem cells [19]. The epithelial cell population could be exclusively isolated from the amnions of term human placentae by specific enzymatic digestion [41]. It has been shown that cultured AECs secrete various morphogens and growth factors, such as epidermal growth factor, Noggin, Activin [42], platelet-derived growth factor, vascular endothelial growth factor, angiogenin, transforming growth factor-beta-2 (TGF-β2), and tissue inhibitors of metalloproteinases (TIMP-1 and TIMP-2) [43].

AECs express surface markers commonly found in embryonic stem cells, such as SSEA-3, SSEA-4, TRA1–60, TRA-1–81 and pluripotent stem cell-specific transcription factors like Oct-4 and Nanog, fibroblast growth factor 4 (FGF4), Rex-1, cryptic protein (CFC-1), and prominin 1 (PROM-1) [33, 44, 45]. AECs express the non-polymorphic, non-classical human leukocyte antigen (HLA-G) which is involved in the induction of immune tolerance. Therefore, the risk of rejection or immune reaction will be reduced upon transplantation of AECs [46, 47]. AECs express secretory leukocyte proteinase inhibitor (SLPI) and elafin which have anti-inflammatory properties. In addition, they both are a part of amnion's innate immune system which prevents infection. AECs also produce β -defensins that are anti-microbial peptides [48, 49].

Tehrani et al. reported for the first time that the AM cells contain cathelicidin LL-37 as a polypeptide involved in innate antibacterial property which can be augmented in the cells after exposure to inflammatory signal IL-1β [50]. Another merit of AECs is the number of cells derived from a single source of tissue. The average yield is reported to be more than 100 million cells per amnion [35].

AECs and AMCs possess unique characteristics which make them an excellent candidate for clinical applications. These cells exhibit low immunogenicity that protects them against the host immune system [51]. Although AECs decrease angiogenesis, AMCs are able to improve angiogenesis via expression of some angiogenic components, such as interlukin-6 (IL-6), growth related oncogene (GRO), monocyte chemoattractant protein-1 (MCP1), C-X-C Motif Chemokine Ligand 8 (CXCL8), intracellular adhesion molecule (ICAM) and migration inhibitory factor [40].

There is no evidence of tumorigenic behaviors of amniotic-derived stem cells. Furthermore, the AM has the merits of decreasing loss of protein, electrolytes, and fluids, reducing the risk of infection, minimizing pain, speeding up the process of wound healing and appropriate handling properties [52].

The potential of human amniotic membrane-derived stem cells for the treatment of hepatic disorders has also been examined. The capacity of these cells to differentiate into liver cells was evaluated, using periodic acid-Schiff staining on amniotic membrane cells in order to evaluate glycogen storage. The positive staining demonstrated that the amniotic membrane cells were able to carry on important physiological function of hepatocytes such as glycogen storage [53].

Takashima et al. have found that several hepatic genes are expressed in AECs, namely: albumin, α1- antitrypsin, cytokeratin-18, glutamine synthetase, carbamoyl phosphate synthetase I, phosphoenolpyruvate carboxykinase, cytochrome P450 2D6 and 3A4 (genes involved in drug metabolism). Hepatocyte nuclear factor 3γ (HNF-3γ), one of the isoforms of transcription factor HNF-3 and CCAAT-enhancerbinding protein-α (C/EBP-α) are genes involved in the regulation of the process of transcription in hepatocytes and have been identified in amniotic cells. The HNF-3γ gene induces the expression of albumin, α-fetoprotein, α1-antitrypsin and transthyretin. On the other hand, the C/EBP-α gene controls glycogen storage and the gene expression of albumin, α-fetoprotein, transthyretin, and tyrosine aminotransferase [36].

The capacity of human AECs to differentiate into type II pneumocytes has also been explored in several studies. One of the studies that evaluate the effect of human AECs in lung injury induced lung inflammation and fibrosis in a mouse model with bleomycin, treating the mice with a human AECs transplant. After the transplant, human AECs were

able to differentiate into phenotypic alveolar epithelium and secrete surfactant protein. The application of human AECs also helped fight lung fibrosis, with a reduction of lung collagen, and inflammatory and fibrotic cytokines [54].

The amniotic cells exhibit some specific cardiac transcription factors, namely cardiacspecific transcription factor GATA4, cardiac-specific genes, such as myosin light chain (MLC)-2a, MLC-2v, cTnI and cTnT, α-subunits of the cardiac-specific L-type calcium channel (α1c) and the transient outward potassium channel [24]. Co-culture experiments have confirmed that amniotic cells have the ability to integrate into cardiac tissue and to differentiate into heart cells. *In vivo* studies have also demonstrated cardiomyocyte differentiation after the injection of amniotic cells into scar tissue post-myocardial infarction [55].

Toda et al. have shown that induction *in vitro* with BMP-2 leads to expression of collagen II and aggrecan. Amniotic cells were implanted into non-cartilage tissue in an animal model with BMP-2 or were implanted with a collagen scaffold into defects generated in rat bone. As a result, morphological changes with the deposition of collagen type II were observed [24].

Wei et al. verified the expression of insulin mRNA in cultivated AECs and the normalization of blood glucose values for several weeks after the implantation of amniotic cells into streptozotocin-induced diabetic mice [56].

Studies evaluating the effect of ASCs in new therapies have been geared toward their utility in several neurological disorders including Parkinson's disease, stroke, traumatic brain injury, and spinal cord injury [57]. The expression of acetylcholine, catecholamines, dopamine, neurotrophic factors, activin and noggin has been found in epithelial and amniotic basement membrane cells [42, 58]. Neural grafts with amniotic epithelial cells in animal models of Parkinson's disease result in the synthesis and release of catecholamine and neurotrophic factors, such as nerve growth factor, neurotrophin-3 and brain-derived neurotrophic factor [24, 59].

In another experiment, human AECs were transplanted into a hemorrhagic stroke model in rats, improving motor skills, and reducing cerebral edema, with survival of transplanted cells in the lateral ventricular wall at 4 weeks [60].

Biological properties of amniotic membrane

Human AM has proven to be an outstanding scaffold for tissue engineering owing to its ability to allow the transport of water and the presence of growth factors such as the epithelial growth factor [61]. Human AM has advantageous characteristics including promotion of epithelization, anti-inflammatory effects, anti-bacterial properties [62], anti-fibrotic properties [19], low immunogenicity as well as immunomodulatory properties [62, 63], anti-angiogenic and non-tumorigenic properties [63].

Basement membrane of AM is composed of collagen type IV, V and VII which facilitates the growth of epithelial cells [18]. Human AM promotes epithelization by excreting EGF, IL-8, insulin-like growth factor 1 (IGF-1), PDGF bFGF, HGF, TGF-b, and other factors that support epithelization and differentiation of different cells [64-66]. Molecules of human AM extracellular matrix, such as fibronectin, laminin-1, laminin-5, collagen type-I, III, IV, V, and VII, also promote cell adhesion and migration [27, 67].

Although the immunogenicity of the AM is controversial, in general, it is believed that the AM possesses low immunogenicity because AECs do not express HLA-A,-B,-D and-DR antigens on the cell surface, but express HLA-G [68]. Presence of interferon – and other immunologic factors has also been observed in the amniotic membrane. It seems that amniotic membrane may induce immunologic reactions in the presence of viable epithelial cells [69]. Human AECs and human AMSCs express low to moderate levels of major histocompatibility complex class I (MHC1) molecules – human leukocyte antigen (HLA), including antigens Ia (HLA-A, B, C) and Ib (HLA-G, E). Moreover, they do not express (or express only very low levels of) HLA II class molecules (HLA-DP, -DQ, -DR) and costimulatory molecules (CD80, CD86) on the cell surface. These properties of hAM decrease the possibility of transplant rejection, which is an important advantage when choosing materials for use in regenerative medicine [51, 70]. In addition, it is generally thought that the immunogenicity of cryopreserved AM tissue is less than that of fresh AM tissues and that cryopreserved cells are expected to be nonviable. This approach guides some researchers to use cryopreserved AM instead of fresh AM [51, 63].

Tissue engineered constructs often provoke an inflammatory reaction known as a foreign body reaction upon implantation. These implanted materials can be degradable or non-degradable. While inflammation can be good in some instances to trigger the healing of an injury, it can also lead to implant failure [71]. There are several reports of the AM reducing inflammation. The AM stromal matrix markedly suppresses the expression of the potent pro-inflammatory cytokines, IL-1α and IL-1β [72]. Matrix metalloproteases (MMPs) are expressed by infiltrating polymorphonuclear cells and macrophages. Natural inhibitors of MMPs have been found in the AM [73, 74]. Hyaluronic acid is a high-molecular-weight glycosaminoglycan that exists in large quantities in the AM and acts as a ligand for CD44, which is expressed on inflammatory cells and plays an important role in adhesion of inflammatory cells, including lymphocytes, to the AM stroma [75]. It has been proved that IL-10 can suppress the effect of pro-inflammatory cytokine IL-6 and tumor necrosis factor-alpha [76]. The IL 10 also suppresses the production of IL-8, a pro-inflammatory chemokine which attracts migration of neutrophils [77]. AM also contains inter-α-trypsin inhibitor which also possesses anti-inflammatory action [78]. AM can also suppress the staffing of inflammatory cell, such as polymorphonuclear cells, CD3 cells, CD4 cells, T cells and CD11b cells to the wounded site thus decreasing inflammation [56, 75].

AM contains natural antimicrobial molecules which are component of innate immune system thus act as safeguard against Gram-negative and Gram-positive bacteria, viral and fungal infection [49, 79]. The innate immune system has evolved to eliminate microorganisms upon entry into

the tissues, creating antigens necessary to produce an adaptive immune response. AECs also have the ability to produce βdefensins [49]. The β-defensins are an important group of antimicrobial peptides which resist microbial colonization, expressed in AECs. Beta 3-defensin is the most prevalent defensin of AECs [80]. Some other important antimicrobial components expressed in AM are low molecular mass elastase inhibitor, secretory leukocyte proteinase inhibitor and elafin [81]. In addition to their anti-inflammatory properties, elafin and secretory leukocyte proteinase inhibitor have antimicrobial actions and act as components of the innate immune system to protect related surfaces from infection [48].

Kim et al. showed that histones H2A and H2B, which possess antimicrobial and endotoxin-neutralizing activity, were localized in the cytoplasm and on the extracellular surface of human AECs [82].

Foreign body reactions evoke stimulation of giant cells and macrophages that produce cytokines and attract fibroblasts, leading to fibrosis. These fibroblasts are activated by the transforming growth factor (TGF)β [71]. The AM downregulates TGF-β and its receptor expression by fibroblasts and in doing so, reduce the risk of fibrosis [83]. Human AM reduces the risk of scarring and adhesion due to secretion of TIMP-1, -2, -3, and -4, which reduce proteases activity on the site of application [74].

Angiogenesis is the formation of new blood vessel from pre-existing one. Some specific compounds have been detected in AM which can prevent angiogenesis. Anti-angiogenic chemicals identified in both epithelial and mesenchymal cells of AM are thrombospondin-1, endostatin and all four types of tissue inhibitors of metalloproteases (TIMP-1, 2, 3 and 4) [73]. Thrombospondin-1 is a potent anti-angiogenic chemical produced by only 20% of mesenchymal cells, whereas endostatin is a powerful anti-angiogenic and endothelial cell growth inhibitor. MMP-1, MMP-2, MMP-3, MMP-4, IL-1 receptor antagonist, collagen XVIII and IL-10 are some of the proteins found in AM and that might have anti-angiogenic activity [73]. Pigment epithelium-derived factor (PEDF) expressed in the AM plays a major role in eliciting the anti-angiogenic activity of AM [84].

Another critical part of safety evaluation is to identify whether human AESCs have an effect on the tumor generation and promotion. In previous studies, the non-tumorigenicity of AECs has been confirmed in many species, including in humans [85-88]. Human AECs and human AMSCs or their conditioned medium (culture medium, which was in contact with hAM or human AM-derived cells during culture) are capable of inducing apoptosis in several cells lines (HeLa cervical cancer cells, MDA-MB231 breast cancer cells, hepatocarcinoma cancer cells HepG2, Hep3B2.1-8, HuH7) [89, 90].

Magatti M. et al. [91] have shown that human AMSCs induce the cell cycle arrest of hematopoietic and nonhematopoietic cancer cells in co-culture by inhibition of positive regulators of the cell cycle (cyclins, cyclin-dependent kinases, mini-chromosome maintenance complex, proliferating cell nuclear antigen) and upregulation of cell cycle inhibitors (cyclin G2, CDK inhibitor 1A, CDK inhibitor N2B). Additionally, Cullin-1 (mediator of ubiquitination and degradation of several proteins, including p21) and RB-1-like protein (p107) are downregulated and retinoblastoma protein (pRB) is upregulated. Consequently, this leads to cell cycle arrest of cancer cells in the G0/G1 phase and prevention of cell cycle progression to S phase.

Mechanical properties of amniotic membrane

Thickness of normal amniotic membrane lies between 0.02 and 0.05 millimeters which includes around 6–8 layers of cells. An average surface area of this membrane is about 1600 square centimeters [92]. The elasticity, stiffness and other biomechanical properties of the ECM depend on the variation in its ingredients, such as collagen, proteoglycan, and elastin [93]. The orientation of the collagen fibrils in the ECM is responsible for the tensile strength, whereas the elastic deformation is related to the presence of elastin fibers [62], laminin, hyaluronic acid, and glycosaminoglycan [94]. Some research has shown the amniotic membrane shear modulus to be between 100 and 400 Pa, with the difference in measurements related to the state of the human AM used [95]. Decellularized human AM presents higher shear modulus than native one, because the denudation process dehydrates the membrane and thus decreases its thickness. It has also been shown that the elastic modulus decreases with increasing human AM thickness [95, 96]. Moreover, the reported values of elastic modulus differ between human AM from distal (135 Pa) or proximal parts (62 Pa) in the placenta [95], which could be attributed to the thickness of the membrane, the tissue composition, and to a time-dependent viscoelastic property of creep of the human AM (tab. 1). This viscoelastic property is related to the increase in the amniotic fluid volume and fetal development during gestation [62].

Table 1. Mechanical properties (intact AM)[97]

Amniotic membrane features	Contributing factors	References
• Direct tensile mechanical properties	Placental: Force before rupture: 1.2 ± 0.2 N Strain at break: 19% ± 3% N Peripheral: Force before rupture: 0.68 ± 0.08 N Strain at break: 16% ± 1% N	Litwiniuk et al., 2017
• Young's modulus	2.29–3.6 MPa	Niknejad et al., 2008
• Tensile strength	5.475 ± 0.135 MPa	Cai et al., 2015;
• Elastic modulus	4.048 ± 1.702 MPa	Ramesh et al., 2017.

Conclusions

The human amniotic membrane, the cellular compounds and extracellular matrix have a lot of benefic proprieties that are/or could be used in treatment of many human diseases.

AM satisfies most of the criteria as an ideal biological tissue and shows almost zero rejection phenomenon.

Taking into consideration the strength, biological properties and grown factors of amniotic membrane it may be a source for embedding therapy treatment.

Biomechanical and good biodegradable properties of human amniotic membrane make it possible to be used for various medical purposes including the manufacture of threads for acupoint embedding therapy and for treatment of peripheral nerve disorders.

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Ethics approval and consent to participate
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Conflict of Interests
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