Review Article

Human Amnion Membrane: Potential Applications in Oral and Periodontal Field

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Abstract

Human amniotic membrane (HAM) is derived from the fetal membranes which consist of the inner amniotic membrane made of single layer of amnion cells fixed to collagen-rich mesenchyme attached to chorion. HAM has low immunogenicity, anti-inflammatory properties and their cells can be isolated without the sacrifice of human embryos. Amniotic membrane has biological properties which are important for the experimental and clinical applications in managing patients of various medical specialties. Abundant, natural and wonderful biomembrane not only protects the foetus but also has various clinical applications in the field of dermatology, ophthalmology, ENT surgery, orthopedics and dental surgery. As it is discarded post-partum it may be useful for regenerative medicine and cell therapy to treat damaged or diseased tissues.

Keywords: *Biomembrane, human amnion membrane, placental allografts, scaffold, tissue engineering*

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Introduction

The amnion has been ascribed to function as its Greek name suggests – as a membranous sac that contains the conceptus and the amniotic fluid. In primates, including humans, it is an adjustable biocontainer that provides the fetus a limited space to allow movements. The amnion is a metabolically active membrane that is involved in solute and water maintaining amniotic fluid homeostasis.

Amniotic membrane or amnion is the innermost layer of the placenta and consists of a thick basement membrane and an avascular stromal matrix. Human amniotic membrane (HAM) has been used successfully for over a decade for a wide range of surgical applications. The use of fetal membrane in skin transplantation was first reported by Davis in 1910. The use of HAM as a surgical wound dressing in the treatment of leg ulcers and ear surgery has been described earlier.[1,2]

HAM contains two cell types, from different embryological origins, which display some characteristic properties of stem cells. Human amnion epithelial cells (hAECs) are derived from the embryonic ectoderm, whereas human amnion mesenchymal stromal cells (hAMSCs) are derived from the embryonic mesoderm. Both populations have similar immunophenotype and multipotential for *in-vitro* differentiation into major mesodermal lineages. The amniotic membrane secretes nutritious factors[3] and suppresses the semiallogenic immune response against the fetus.[4-6]

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The thickness of the human term amnion varies among individuals and depends on the location of the sample (70–180 μm thick), however, it is remarkably strong and elastic. Amnion withstands the progressive stretching of the growing embryo, internal and external traumas, and fast and slow pressure changes. In a vast majority of the amniotes, amnion is one of the very few tissues that has no vascularity. In humans, the chorion and the amniotic fluid transfer nutrients to the avascular amnion by diffusion.[7] Amniotic membrane has an excellent candidature to be used as a native scaffold for tissue engineering, and in addition, maybe easily obtained, processed, and transported.[8]

Historical use of placental allografts

The first recorded clinical use of amnion tissue was for use in skin transplantation in 1910.[1] Shortly thereafter, it was frequently used to treat ulcerated skin conditions,[9] econstruction of vaginal malformed organs,[10] and vestibuloplasty.[11]

The clinical use of cyropreserved amnion allograft in ophthalmic surgery was first reported in 1997.[12] Today these allografts are commonly used in ophthalmic surgery, and

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literature suggests that both cyropreserved amnion allograft[13] and dehydrated amnion allograft[14] provide results equivalent to conjunctive autograft tissue. It has been used as an adhesion barrier in spine and orthopedic procedures as well as in the treatment of chronic wounds.

Anatomy and histology of the amniotic membrane

Amniotic membranes develop from extraembryonic tissue and consist of a fetal component (the chorionic plate) and a maternal component (the deciduas).

Scientific basis of clinical application

Amniotic membrane is a gift of nature which not only protects the fetus inside the womb but also has several medicinal properties. It serves as a natural barricade to protect the fetus from bacterial infection and trauma.[15] Amniotic membrane acts as a scaffold for proliferation and differentiation due to the presence of fibronectin, elastin, nidogen, collagen types I, III, IV, V, and VI, elastin, and hyaluronic acid.[16] Another important advantage of using amniotic membrane in allotransplant or xenotransplant is lack of immunogenicity. Promotion of epithelialization, anti-inflammatory properties, antifibrotic properties, antibacterial properties, and antiangiogenic properties are confirmed by the presence of several related factors that makes amniotic membrane an ideal therapeutic for burns and wound healing. Amniotic membrane is known to promote epithelial cell migration, adhesion, and differentiation, and is also an ideal substrate for supporting the growth of epithelial progenitor cells by prolonging their lifespan. Finally, amnion has been also used as an allograft in general surgery for reconstructions, as an autograft in neonatal reconstruction surgery, and as a scaffold in tissue engineering research.[17-23]

Amnion vs chorion

- Amnion and chorion, both are part of the extraembryonic membranes, which function in an embryo's overall development. They also play important roles in the embryo's nourishment, breathing, and seepage
- The amnion is a thin but tough sac of membrane that covers an embryo. It is present in the embryonic development of reptiles, birds, and mammals. However, it is not present in the development for amphibians and fish offspring
- Amnion is an inner membrane that surrounds the embryo whereas the chorion surrounds the embryo, amnion, and other membranes
- The amnion is filled with amniotic fluid, which holds the embryo in suspension while chorion acts as a protective barrier during the embryo's development
- The amnion comprises of tresodeum and ectoderm while the chorion includes the trophoblast and the mesoderm
- The chorion has a special feature called chorion villi, which acts as a barrier between the maternal blood and fetal blood. It absorbs maternal blood for the embryo's necessities whereas the amnion plays a part during the delivery stage.[24]

Aminotic membrane: Preparation, processing and preservation

Preparation

Fresh membrane is obtained from the placenta at the time of delivery, either vaginal or caesarian section. Robson and Krizekl rinsed the membrane in a 0.025% solution of sodium hypochlorite and stored at 4°C in sterile solution containing penicillin. They showed that membranes remained sterile up to 6 weeks. Dinno *et al*. performed cultures to study the sterilization of amniotic membranes. Preservation with 1:40 dilution of sodium hypochlorite revealed no positive cultures until 30 days.

Processing

For clinical use, amniotic membrane can be prepared in the following forms:[25]

- Fresh membrane
- Dried membrane
- Frozen membrane
- Freeze derived irradiated membrane
- Stabilized amniotic membrane
- Cryopreserved membrane.

Preservation

Glycerol has been used as a cryoprotective agent for a long time. Because of its high osmotic pressure, it extracts interstitial water from the amniotic membrane. In this method, 80% glycerol is used for drying the amniotic membrane, which can thereafter be preserved at 4°C for a long time, although it loses some of its biologic properties. This type of preserved amnion is used for dressing burn wounds.[26-35]

Properties of the amniotic membrane

The human amniotic membrane at term has a number of properties that has made its clinical use a success, which includes the absence of inducing an immune reaction and having an anti-inflammatory effect. Its stromal matrix also shows a marked suppression of proinflammatory cytokines, IL-1α, and IL-1β expression.[36]

Amniotic membrane has also been known to have natural inhibitors of MMPs[37] and hyaluronic acid, which is a higher molecular-weight glycosaminoglycan and acts as a ligand for CD44 to the amniotic membrane stroma.[38] The amnion has been described as antiangiogenetic and bacteriostatic, as well as having analgesic properties. It promotes re-epithelization and prevents scarring and functions as an evaporation barrier.[39,40]

The human amnion possesses low or no immunogenicity. Cells from the fetal membranes having immunomodulatory properties may be involved in the maintenance of fetomaternal tolerance.[41] It has been shown that the human leukocyte antigens (HLA) class I are expressed in amniotic epithelial and mesenchymal cells whereas HLA class II antigens are not synthesized in the cells of the amniotic membrane.[42] Epithelial and mesenchymal amniotic cells secrete a number of anti-inflammatory proteins such as Activin A, IL-1 receptor antagonist (IL-1ra), and IL-10, which are deposited

within the amniotic membrane stroma.[43,44] Suppression of proteinase and MMP activation by amniotic membrane leads to decreased infiltration of inflammatory cells. Moreover, proapoptotic activity of the amniotic membrane has also been reported – amnion can promote the apoptosis of leucocytes.[45] Amniotic epithelial cells express the apoptosis-inducing genes Fas L, TNF, and TRAIL. Human amniotic membrane has been shown to be equally effective as autologous skin grafts, but superior to allo and xenogenic skin grafts for decreasing bacterial counts in open granulating rat wounds.[46] Kanyshkova *et al*. reported the presence of the antibacterial protein lactoferrin in the membrane.[47]

Amniotic membrane is one of the very few human tissues that are completely avascular, hence, its ascribed antiangiogenic properties. Antiangiogenic factors (endostatin, TSP-1, and TIMPs) are produced within the amnion, however, angiogenic factors such as VEGF and bFGF have been also shown to be present in amniotic membrane.[48] Depending on the setting of *in vitro* and *in vivo* experiments with amnion or amnion-derived cells, either suppression[49] or promotion of neovascularization has been reported.[50]

Amniotic membrane tissue has antimicrobial activity. Aminiotic tissue produces β-defensins, which is a major group of antimicrobial peptides that are expressed by epithelial cells and form an integral part of the immune system. They protect epithelial surfaces from microbial colonization. Amniotic tissue also produces secretory leukocyte proteinase inhibitor (SLPI) and elafin. In addition to their anti-inflammatory properties, elafin and SLPI both have antimicrobial actions and act as components of the immune system to provide protection from infection. Amniotic membrane treatment with both the lactoferrin and interleukin-I receptor antagonist make the amniotic membrane antimicrobial as well as anti-inflammatory. Lactoferrin, a global multifunctional protein, has both antimicrobial as well as anti-inflammatory effects, which serves as an antioxidant and iron chelator in tissue. It is also known to suppress the production of IL-6 in the amniotic fluid during amniotic infection.

Periodontal plastic surgical procedures are aimed at coverage of exposed root surface. Owing to the second surgical donor site and difficulty in procuring a sufficient graft for the treatment of root coverage procedures, various alternative additive membranes have been used. A recent resorbable amniotic membrane not only maintains the structural and anatomical configuration of regenerated tissues but also enhances gingival wound healing and provides a rich source of stem cells. Therefore, amniotic membrane is a material of choice these days in augmenting better results in various periodontal procedures.[51]

Mechanical properties

Differentiation of some progenitor cells depends on mechanical stimulus/signals, therefore, a scaffold must create an adequately stiff environment throughout the site where new tissue is desired. Increase in stiffness enhances the stability of scaffold and prevents displacement that leads to uninterrupted healing as well as feasibility in exchange of metabolic products of involving cells during the early phase of healing. Scaffold should also have sufficient elasticity for maintaining the shear stresses of surrounding tissue. Collagen and elastin in extracellular matrix provide stiffness and elasticity for amniotic membrane, respectively. The mechanical response of amniotic membrane is time dependent that is termed as viscoelastic in nature.

Amniotic membrane is a semipermeable membrane and is an immunotolerant structure. The amniotic membrane fulfills the current mechanical concept of guided tissue regeneration (GTR), which amends it with the modern concept of biological GTR. Biomechanical GTR proposed herein using amniotic membrane not only maintains the structural and anatomical configuration of regenerated tissues but also contributes to the enhancement of healing through reduction of postoperative scarring and subsequent loss of function, providing a rich source of stem cells. Amniotic membrane enhances gingival wound healing properties and reduces scarring. Excellent revascularization of the amniotic membrane is another favorable property. Amniotic membrane is potentially a good grafting material with very good wound coverage. It enhances wound healing process, good postoperative function, and esthetics without any complications. HAM could be one of the considered options in the reconstruction of oral cavity defects because it ensures good reconstruction, postoperative function, and esthetics.

The dehydrated amnion/chorion membrane allograft can also be micronized, which allows it to be administered as a topical powder or mixed with saline to create an injectable solution or a topical gel. Use of amniotic membrane has recently increased clinically as an allograft material for chronic and acute wound care management, for scar tissue reduction, as a barrier membrane, and as a soft tissue regeneration graft. Amniotic membrane is highly useful and effective as a culture substrate.[52]

Stem cell characteristics of amnion-derived cells

Stem cell therapy is emerging as a powerful tool to generate biological substitutes and regenerate for damaged tissue with high proliferability, differentiability, and function. The incorporation of these cells in the periodontal wound may, therefore, accelerate periodontal healing. Many efforts are under way to develop novel bioengineered wound-healing products, including involvement of mesenchymal stromal cells (MSCs) in the wound-healing process.

At present, tissue engineering uses human embryonic stem cells (HESCs) as allogenic cells. The HESC lines have been derived from the inner cell mass of blastocyts that are 3–5 days old, originally described by Thomson *et al*.[53] the ongoing researches have suggested that the amniotic epithelium retains the reservoir of stem cells throughout the pregnancy as the researchers have successfully generated ectodermal, endodermal, and mesodermal cell lineages using HESCs.[54,55]

The current understanding of pluripotency is based on extensive studies of mouse and HESCs, and more recently also induced pluripotent stem (iPS) cells.[56,57] In 2004,

Tamagawa *et al*. reported the isolation of a pluripotent stem cell line derived from cultured whole HAM. These stem cells contributed to the formation of chimeric mouse/human embryoid bodies *in vitro*, giving rise to cells with characteristics of the primordial liver, lung, and digestive tract, but also to neural, epithelial, and hematopoietic cells, and blood vessels. Human amnion-derived cells seem to give rise to cells of all three germ layers.[58]

Amniotic membrane for potential use in tissue engineering

An important component of tissue engineering is the supporting matrix upon which cells and tissues grow, also known as the scaffold. Scaffolds must easily integrate with host tissue, and provide an excellent environment for cell growth and differentiation. Most scaffold materials are naturally derived from mammalian tissues. The amniotic membrane is considered an important potential source for scaffolding material.

Amniotic membrane has biocompatibility, low immunogenicity, adequate mechanical properties (permeability, stability, elasticity, flexibility, resorbability), good cell adhesion, and easy delivery of biomodulatory agents such as growth factors and genetic materials. The attachment of a cell to a scaffold is largely affected by the components of the scaffold's extracellular matrix. The presence or absence of certain extracellular matrix molecules such as collagen, laminin, fibronectin, and vitronectin within any basement membrane has a huge influence on the adhesion and growth of the overlying stem cells. In addition to allowing the cells to attach and migrate, the extracelllar matrix molecules also serve as adhesion ligands, which transmit signals *via* their interaction at cell surface receptors. When epithelial and mesenchymal cells are seeded on a cellular scaffold created from the amniotic membrane, the cells were highly interconnected and capable of penetrating the porous structure of the amnion scaffold. Cultivation and seeding of epithelial cells on an amnion scaffold is a frequently used method for ocular surface and skin reconstruction.[48-50] And finally, cultivation of endothelial cells on an amniotic membrane scaffold has also been reported as a potential approach for vascular TE.

Biocompatibility is said to be the property of being biologically compatible by not producing a toxic, injurious, carcinogenic, or immunological response in living tissue, and is also a major prerequisite for choosing a scaffold.[59,60] In addition, their mechanical properties should include permeability, stability, elasticity, flexibility, plasticity, and resorbability at a rate congruent with tissue replacement.[61] Scaffolds should also allow cell adhesion and the potential for delivery of biomodulatory agents such as growth factors and genetic materials.[62-64]

Clinical applications of human amnion

Human amnion has a long history of clinical applications. It was reported for the first time as a biological dressing to heal skin wounds a century ago. In the management of open wounds, the major goal is to obtain a clean and closed wound in the shortest time possible, thereby preventing fluid, heat, and nutrient loss as well as wound infection, pain, and decreased mobility. Amniotic membranes are efficiently used as allografts for treating skin burns; open and nonhealing ulcers; pressure sores; and surgical, infected, and traumatic wounds.[65,66] An alternative treatment to manage wounds in the oral cavity, such as the tongue, buccal mucosa, vestibule, palatal mucosa, and floor of the mouth; in the reconstruction of the oral cavity, bladder, and vagina; tympanoplasty; arthroplasty, and so forth. Its adhesive and tight contact with the injured surface promotes hemostasis and good pain relief due to exposition of nerve fibres. Good biocompatibility and mechanical properties such as permeability, stability, elasticity, flexibility, plasticity, and resorbability also make it a promising scaffolding material in tissue engineering as in cell adhesion, and the potential for delivery of biomodulatory agents such as growth factors and genetic materials. Anti-inflammatory and antiscarring property of amniotic membrane have shown decreased necrosis and rapid healing of ulcers with herpes simplex virus (HSV), varicella zoster virus infected tissues, erythema multiforme major (Stevens–Johnson syndrome), and cervical necrotizing fasciitis. HAM has been tried in the reconstruction of temporomandibular joint ankylosis because it prevents fibrosis and reankylosis when used as an interpositional material. Amniotic membrane is even used as a carrier for local delivery of various drugs such as antibiotic netilmycin (NTM) and antiviral drugs such as acyclovir (ACV) and trifluridine (TFU). Amnion has been tried as a graft material after vestibuloplasty where it prevents secondary contraction after surgery and maintains postoperative vestibular depth.[67-78]

Current clinical uses of amniotic tissue in dentistry

Amniotic membranes have already been used extensively as biologic dressings in ophthalmic, abdominal, and plastic surgery. The laminin structure of amnion tissue is nearly identical to that of native human tissue such as oral mucosa. Reconstruction of a buccal mucosal defect after excision of speckled leukoplakia using HAM has been reported with a promising result.[79]

Contemporary dental implant treatment recommends that at least 1 mm of bone surrounds all aspects of the implant fixture. To achieve such a goal, the concept of site preservation is frequently employed. A resorbable amnion chorion membrane has recently been introduced as a new barrier for site preservation. Unlike cadaveric allograft, xenograft, and alloplast barrier membranes, placental allografts are composed of immunoprivileged tissue, possess antibacterial and antimicrobial properties, reduce inflammation at the wound site, and provide a protein enriched matrix to facilitate cell migration.[80]

A novel allograft composed of amnion tissue has recently been introduced for periodontal plastic surgery. Collected data and subjective observation by the authors indicate that the use of processed dehydrated allograft amnion provides good results in terms of root coverage, increased tissue thickness, and increased attached gingival tissue. Processed dehydrated allograft amnion demonstrated excellent esthetic results in terms of texture and color match without postoperative

discomfort and adverse reactions.[81] The allograft has been reported to treat Grade II furcation defects with DFDBA and xenograft by guided tissue regeneration.[82]

Ambio5™, a 3rd generation amniotic membrane, was developed to further optimize and simplify amniotic membrane transplantation to yield a substantially thicker, more intact, and native amniotic membrane allograft.

The ability of processed dehydrated allograft amnion to self-adhere eliminates the need for sutures. The procedure becomes less technically demanding reducing the surgical time. The ability to self-adhere makes processed dehydrated allograft amnion an attractive option for multiteeth procedures and recession defects in particularly posterior region. Processed dehydrated allograft amnion may provide an effective alternative to autograft tissue in the treatment of shallow-to-moderate Miller Class I and II recession defects.

The clinical usefulness of the hyperdry amniotic membrane as an intraoral wound-dressing material has been studied, and the results suggest that the hyperdry amniotic membrane is biologically acceptable to oral wounds and could be a suitable clinical alternative for the repair of the oral mucosa.[83] A successful closure of oronasal fistulas was observed in minipigs using interposed grafts of cryopreserved HAM, offering a simple and effective technique for tension-free closure of such fistulas.[84]

The future of amniotic tissue

The benefits of novel allograft include reduction of surgery time, improving patient outcomes with an affordable price tag. Amnion tissue has many potential uses across the field of medicine and dentistry. To treat gingival recession and a membrane barrier for guided bone and guided tissue regeneration, the technology has tremendous potential wherever there is mucosal tissue. Third generation amniotic membrane has been developed to further optimize and simplify amniotic membrane transplantation for ophthalmic and dental surgery as well.

Conclusion

The safety, logistical, and surgical advantages of amnion membrane are vast. Dental applications of amniotic membrane are currently showing great promise in various specialties of dentistry. Amniotic membranes have already been used extensively in medical field as biologic dressings in ophthalmic, abdominal, and plastic surgery. Amniotic membranes have a rich inheritance of collagen types I, IV, V, and VI, proteoglycans, laminin, and fibronectin. Collagen is well tolerated and bioabsorbable, has hemostatic properties, and encourages migration of adjacent autogenous connective tissue and epithelial cells over its surface. Laminins exhibit a variety of biological activities including promotion of cell attachment, growth, and differentiation of number of cell types. Fibronectin is involved in many cellular processes including tissue repair, blood clotting, cell migration, and adhesion. The use of this novel biological membrane is rising in various fields of tissue engineering, medicine, regeneration biology, and stem cell research. The clinical application of amniotic membrane not only maintains the structural and anatomical configuration of regenerated tissues, but also contributes to the enhancement of healing through reduction of postoperative scarring and subsequent loss of function, providing a rich source of stem cells. Other properties of the AM include anti inflammation, antifibrosis, antiscarring, antimicrobial, low immunogenicity, and reasonable mechanical property, which are all important for use in tissue engineering. However, further research and long-term clinical trials investigating the full potential of this stem cell reservoir are still warranted to strengthen the fact that amniotic membrane is indeed a reservoir for regeneration.

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Conflicts of interest

There are no conflicts of interest.

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