Review article

Amniotic Membrane: An Innovative Material for Repair and Regeneration in Oral and Maxillofacial region- A Review

Thapliyal GK, Kumar V, Gour S

Abstract: Amniotic membrane is the inner most lining of the human placenta that is normally discarded after parturition. The membrane has numerous growth factors, proteins and stem cell reserves that help in accelerating wound healing with regeneration of the lost tissues. With improvements in the processing and storage technologies, amniotic membrane has found application in various fields of surgery. Recently, this tissue has found application in the field of maxillofacial surgery, because of its inherent wound-modulating properties. This review paper discusses the structure, properties, mechanisms and the applications of this neglected tissue that makes it a potential material for regeneration especially in the field of oral and maxillofacial surgery.

Keywords- Amniotic membrane, growth factors, stem cells, maxillofacial surgery.

INTRODUCTION

Regenerative medicinal therapy has emerged as a powerful tool to generate biological substitutes and repair damaged tissues, by either transplanting exogenous or stimulating endogenous stem cells with highly proliferative, differentiability, to restore proper function and provide better aesthetics. Mesenchymal stem cells (MSC) are one of the major cell populations, to mediate regeneration due to their multipotential properties,[1] They can be obtained from various tissues such as bone marrow, periosteum, peripheral blood, adipose tissues and skin. MSC obtained from these tissues have a few limitations, relatively invasive procedure and poor quality of cells if taken from elderly or medically compromised patients.[2] Therefore newer developments in tissue engineering are focused on alternate sources of MSC, to provide adequate amount of stem cells with minimal patient morbidity.

Amniotic membrane of the human placenta is a rich source of stem cells and studies have shown that human amniotic mesenchymal cells (hAMSC) are non tumorigenic, have anti-inflammatory and low immunogenicity effects and are capable of differentiating into three forms of germ layers.[3-7] Advantages of hAMSC over autogenously derived stem cells are no morbidity in its procurement procedures, unlimited amount available[8] and isolation of hAMSC does not sacrifice the embryo, as in the case of embryonic stem cells, thus no legal or ethical issues arise.[9] Based on these factors amniotic membrane (AM), can be preserved and used as a source of stem cells in various tissue regenerative modalities. Here we present an overview of its inherent structure, properties, processing methods and clinical applications in maxillofacial surgery.

The review was based on 3 electronic databases (MEDLINE, EMBASE, Cochrane) for articles published from 1910 to 2015. In addition, selected journals were searched by hand for relevant articles, on human amniotic membrane and its application in maxillofacial surgery.

History- Placental tissues are being used, for the treatment of wounds and reconstructive procedures since the early 20th century. Davis in 1910, first reported the use of fetal membrane as skin substitutes for treating open wounds.[10] later it was advocated in the management of conjunctival defects, burns and skin ulcers.[11] But due to lack of proper sterilization protocols and storage procedures, its use was diminished. In 1965, Dino et al demonstrated how to separate, sterilize and store AM.[12] In maxillofacial surgery, Lawson first used AM with PMMC flap for closure of oral defects in 1985.[13] Lai et al, in 1995 used single layer of amnion in treatment of OSMF. AM was also used in vestibuloplasty, oronasal/oroantral fistulas and as an

interpositional material in TMJ ankylosis cases.[14]

Structure-Amniotic membrane develops from extra-embryonic tissues and consists of a foetal component (the chorionic plate) and a maternal component (the deciduas). These two parts are held together by the chorionic villi and connect the cytotrophoblastic shell of the chorionic sac to the decidua basalis. The foetal component, which includes the amniotic and chorionic foetal membranes, separates the foetus from the endometrium. The amniochorionic membrane forms the outer limits of the sac that encloses the foetus, while the innermost layer of the sac is the AM. The AM consists of an epithelial monolayer, a thick basement membrane, and an avascular stroma (Fig 1).

The AM contains no blood vessels or nerves, thus its nutrition is supplied directly by diffusion out of the amniotic fluid and/or from the underlining decidua. The innermost layer, nearest to the foetus, is called the amniotic epithelium and consists of a single layer of cells uniformly arranged on the basement membrane. The compact layer of stromal matrix adjacent to the basement membrane forms the main fibrous skeleton of the AM. 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 intermediate layer (spongy layer or zona spongiosa) 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.[15]The spongy layer is loosely connected to the chorionic membrane, hence the AM is easily separated from the chorion by means of blunt dissection.

Properties- Several features of AM, allows it as an ideal material of choice in maxillofacial surgeries:

A) Low Immunogenicity- Any graft material should be immunologically inert with reduced risk of rejection on transplantation. In AM the amniotic epithelial cells expresses only non-polymorphic, non-classical human leukocyte antigen (HLA-G) and not HLA-A,-B,-D and –DR on their surfaces.[16] These MSC show low allogeneic reactivity, because of lack of expression of CD80 and CD8 molecules. Also they suppress T cell, dendritic cell and B cell function, thus rendering AM with immunosuppressive properties.[17,18]

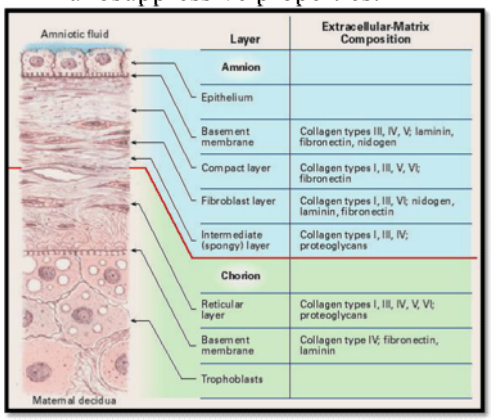

***Figure 1**: Structure of the foetal membrane. Adapted from Parry and Strauss (1998).*

B) Promoting epithelialization- Basement membrane of AM is composed of collagen type IV and V, which facilitates growth and migration of epithelial cells,[19-21] strengthens basal epithelial cell adhesion,[22] promotes differentiation of epithelial cells[23] and prevents apoptotic cell death.[24] These properties helps in treating epithelial defects.

C) Anti scarring and anti inflammatory effects- During wound healing, neutrophils, macrophages and giant cells reach the surgical site to counter invading microorganisms and reduce inflammation. They produce cytokines which attract fibroblasts causing fibrosis. Fibroblasts are activated by transforming growth factor (TGF) beta. AM secretes vascular endothelial growth factor (VEGF), hepatocytes growth factor (HGF) and

maintain the production of TGF-1 and TGF-3 and prevents scarring.[25] AM also reduces the regulation of TGF-beta and promotes tissue reconstruction, rather than scar tissue formation.[26] The MSCs in AM decreases the secretion of cytokines causing inflammation e.g tumor necrotic factor-alpha (TNF-alpha) and interferons (IFN) and increase anti-inflammatory cytokines interleukin (IL)-10, IL-4, IL-alpha and IL-1beta. AM also reduces inflammation by reducing the count of polymorphonuclear cells, CD3 cells, CD4 T cells and CD11b cells, at the surgical site.

D) Increased vascularization- VEGF secreted by AM initiates neovascularization, by releasing insulin derived growth factor (IGF).[27]
E) Antimicrobial effect- AM tightly adheres to the wound by fibrin and elastin linkages and helps in maintaining lymphatics, protects phagocytes from exposure, faster removal of debris and reducing contamination,[28] by secretion of antimicrobial factors LL-37.[29] Beta 3 defensins present in AM, are an important group of antimicrobial peptides, which resist microbial colonization.[30] Other antimicrobial components expressed in AM are low molecular mass elastase inhibitor, secretory leukocyte proteinase inhibitor and elafin.[31]
F) Pain reduction- AM reduces pain post surgically, as it decreases inflammation and enhances healing.[28] It also forms a layer over exposed nerve endings and prevents irritation by external stimuli.
G) Stem cell marker expression- amniotic membrane expresses stem cell markers e.g OCT-4, Sox-2, FGF-4, Rex-1, CFC1, Nanog, DPPA3, PROM1, PAX6, SSEA-3, SSEA-4, Tra 1-60, Tra 1-81, GCTM2, SSEA-3, SSEA-4, OCT-4, Rex-1 and BMP-4,[32,33] which are detected through RT-PCR, FACS and immunocytochemistry. These confirm the clonogenic capabilities of hAMSC.
H) Mechanical properties- AM forms approximately 20% of the chorion amnion layer, but provides major part of stiffness and strength against the hydrostatic pressure of amniotic fluid and prevents premature rupture of foetal membrane (PROM). This property of AM is described as 'viscoelasticity'.[34] Elastin, is the molecular basis of this property, which allows AM to be used as a scaffolding material for various tissue engineering procedures.

Processing and preservation of amniotic membrane- Fresh membrane is obtained from the placenta at the time of delivery in either vaginal or caesarian sections and can be preserved by various techniques, that include hypothermic storage at 4°C, freeze drying by liquid nitrogen at -196°F, γ-sterilization, glycerol preservation and cryopreservation. These processes can alter the viability of cells and growth factors in the AM, for e.g storage in glycerol at 4°C, results in immediate cell death, cryopreservation with di-methylsulphoxide (DMSO) at -80°C causes cell rupture and loss of angiogenic properties.[35] To overcome these problems, a newer preservation technique was employed, freeze dried-irradiated (Lyophilized), here the membrane is first freeze dried at -60°C under vacuum(atmospheric pressure 102mm of Hg) for 48 hours and then irradiated with 2.5 mega Rads (25 K Gray) in a batch type cobalt-60 irradiator.[36] In order to increase the shelf life of AM, it was exposed to far-infrared rays and microwaves. This led to formation of 'Hyper-dry amnion (HAM)[37] which could be stored at room temperature in sterile sealed packs. Even after proper sterilization by any technique, screening test for infectious diseases such as human immunodeficiency virus (HIV) type 1 and 2 antibodies, human T-lymphotropic virus (HTLV) type 1 and 2 antibodies, Hepatitis C antibody, Hepatitis B surface antigen, Hepatitis B core total antibody, serological test for Syphilis, HIV type 1 nucleic acid test, and Hepatitis C virus nucleic acid test is mandatory.[13]

Method of use- The wound should be thoroughly scrubbed with antiseptic solution, all granulation tissue should be removed and complete haemostasis is achieved, to provide a

dry field. Under full aseptic conditions, the membrane is applied to rough (chorionic) surface next to the wound and pressed gently to prevent air bubbles entrapment. A layer of anti-bacterial gauze (Soframycin), is placed over the membrane followed by moist gauze, dry gauze and bandage. Dressing should be changed after every 24 to 48 hours along with the membrane and continued for 7-10 days.[37] Freeze dried irradiated membrane is applied similarly, but before placement it should be soaked in saline for 1-2 minutes.[36]

Apllications in Oral and maxillofacial surgery- Due to its innate property of re epitheliazation and vascularization, AM has proven to be a good dressing material for management of wounds in various regions of oral cavity e.g tongue, buccal mucosa, vestibule, palatal mucosa and floor of the mouth.[38] Rapid healing of ulcers, caused by herpes simplex virus (HSV), varicella zoster virus, erythema multiforme, is seen because of its anti-inflammatory and anti-scarring properties. Epithelial defects, in cases of necrotizing fasciitis have been successfully treated by AM.[11]

HAM was used as a grafting material in repair of oronasal fistulas. The membrane was interposed between the oral and nasal layers and primary closure was done.[39] The study showed effective and tension free closure of the fistula. In oral submucous fibrosis after excision of fibrous bands a single layer of fresh amnion, was grafted over the wound. The graft was carefully contoured to the surgical site to prevent tenting over the defect. Along with good wound contracture, increase in mouth opening was noted on follow-up visits.[40] In patients with deficient depth of the mandibular vestibular sulcus, vestibuloplasty followed by grafting with amnion over the denuded periosteum, showed sufficient covering of the raw surface, and maintainance of the vestibular depth.[41]

The AM being biocompatible and having properties like permeability, elasticity, and ability to resorb, forms a good scaffolding material in tissue engineering procedures and delivery of biomodulatory agents such as growth factors and genetic materials.[42] Amniotic membrane has been used with PMMC flap for oral cavity reconstruction and has shown enhanced re-epithelialization of the oral cavity and reduced contracture.[13]

Controversies and limitations- AM consists of biomolecules, like other biological tissues, these biomolecules are responsible for maintaining homeostasis for fetal growth. Few of these molecules have contradictory properties, for example Anti-inflammatory cytokines IL-1Ra and IL-10 present in AM can suppress inflammation but IL-6 and IL-8 can promote inflammation.[38] Likewise, amniotic membrane harbors various growth factors e.g., EGF, which supports epithelial growth and TGF supports wound healing. But TGF itself enhances formation of scar tissue and becomes contrary to the proposed anti-scarring effect of AM.[43] Also, tissue inhibitors of metalloproteinases (TIMPS), present in AM prevent vascularization.[44]

CONCLUSION: Amniotic membrane transplantation has gained an acceptable position in the surgical armamentarium of Maxillofacial surgery as it ensures a reliable and affordable option for surgeons and patients. Recent advances in tissue preservation techniques have resulted in commercially available amniotic membrane products. But still there is less clinical evidence on effectiveness of these products. Further research and long term prospective randomized controlled clinical trials, and optimization of controversial properties, are advised for investigating the full potential of amniotic membrane.

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