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Human Amniotic Membrane: Can it be a Ray of Hope in Periodontal Regeneration?

*** Dr. Supriya Mishra** | MDS, Department of Periodontology, Maitri College of Dentistry and Research center, Anjora, Durg, Chhattisgarh. * Corresponding author

Dr. Shweta Singh | MDS, Department Of orthodontics, government dental college and research center, Raipur, Chhattisgarh.

ABSTRACT

Periodontal diseases leading to deterioration of tooth-supporting structures are a serious concern for clinicians. Conventional and existing treatment procedures are not predictable and are associated with a relatively high degree of variability and usually insufficient to promote the regeneration of damaged structures. However, in the emerging field of regenerative medicine, the mesenchymal stem cells derived from human amniotic membrane has given us a ray of hope. 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 post-operative scarring and subsequent loss of function and providing a rich source of stem cells.

KEYWORDS periodontal disease, amniotic membrane, periodontal regeneration

INTRODUCTION

Periodontal disease is a chronic inflammatory response that occurs in response to a predominantly gram negative bacterial infection originating from dental plaque.[1] It is characterized by loss of both soft and hard tissues anchoring the tooth and when left untreated; can lead to eventual tooth loss.[2] Conventional treatment, including oral hygiene instructions, and scaling/root planing, aims to prevent the disease, or slow or stop its progress, and maintain the therapeutic goals achieved but is usually insufficient to promote the regeneration of damaged structures.[3] Moreover, outcomes of existing procedures; guided tissue regeneration using non-resorbable and resorbable collagen membranes, Enamel-Matrix Derivatives or Platelet Rich Plasma, are not predictable and are associated with a relatively high degree of variability.[4] In the promising field of regenerative medicine, there are many potential cell sources for regenerative medicine, including bone marrow-derived mesenchymal stem cells, tissue specific progenitor cells, embryonic stem (ES) cells, and induced pluripotent stem (iPS) cells. Although their biological potentials have been demonstrated, none of these cells is widely accepted as a definitive cell source for clinical applications. Each cell type possesses different advantages as well as limitations for their use, such as safety or availability.[5] Hence, with so many developments in our understanding on periodontal regeneration, biologic and materials sciences; complete regeneration still is an unrealistic situation in many clinical situations due to the complexity of the biological events, factors, and cells involved in regenerative process in the periodontium.[6,7] (table 1)

TABLE 1: Complications in the regenerative process in the periodontal environment[7]

1	Microbiota	Diverse pathogenic microbial flora may exert on the outcome of surgical procedures.
2	Multiple specialized cell types	The reformation of lost periodontal tissues and the regeneration of the dentogingival junction necessitate the concerted action of at least 5 cell types from local sites (cementoblasts, osteoblasts, fibroblasts, junctional and sulcular epithelial cells and endothelial cells) and the participation of several different resorptive cells derived from either monocytes circulating in blood or fibroblast populations.
3	Multiple specialized attachement complexes	The cells that synthesize these attachments are required to maintain the dimensions and their specialized type of attachment over very narrow dimensional ranges and to tooth surfaces and gingival tissues that undergo movement with respect to each other.
4	Avascular tooth surface	Exposed root surfaces may act as substrates for cell attachment; they cannot contribute directly to the formation of blood vessels or the other cell types that are essential for reattachment.
5	Stromal cellular tooth surface	The proliferation and appropriate differentiation of the cells participating in wound healing depends in part on contact with stromal elements. When there is extensive loss of stromal tissue, such as in periodontitis, the number of messages lost that is needed to control the differentiated functions of periodontal cells is still unknown.

Mesenchymal stem cells (MSCs) are one of the major cells populations that play an important role in mediating each phase of the wound-healing process: inflammatory, proliferative, and remodeling. MSCs residing within various tissues, including bone marrow and adipose tissue, are reported to differentiate into various types of cells including osteoblasts, chondrocytes, and adipocytes. This multipotency renders MSCs an attractive therapeutic source for regenerative medicine. Many efforts are under way to develop novel bioengineered wound-healing products, and include involvement of MSCs in the periodontal wound-healing process. However, because an invasive procedure is required to obtain autologous bone marrow or adipose tissue-derived MSCs, an alternative source of MSCs that can be obtained non-invasively is desirable. Recently, the fetal derived MSCs from the placenta or other gestational tissues like the amniotic fluid, umbilical cord can emerge as novel materials for periodontal regeneration.[6]

STRUCTURE OF HUMAN AMNIOTIC MEMBRANE

The human placenta is a complex organ that is very important for the development and survival of the fetus throughout the gestation. It is about 10-15 micrometers thick and consists of two fetal membranes; the inner amniotic membrane and the outer chorion. The AM encases the amniotic fluid and fetus, and is highly flexible because of which it is easily be separated from the chorion. The membrane is composed of multiple lay-

ers which include a single layer of epithelial cells, a basement membrane and an avascular connective tissue matrix (fig.1 table 2,3).[6]

Fig.1 Structure of Human Amniotic Membrane (Courtesy-MiMedx Purion® process)

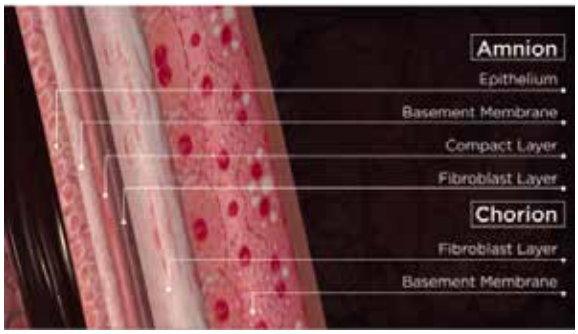

TABLE 2: DIFFERENT LAYERS OF HUMAN AMNIOTIC MEMBRANE

DIFFERENT LAYERS OF AMNIOTIC MEMBRANE[6]		
EPITHELIAL LAYER	BASEMENT MEMBRANE	CONNECTIVE TISSUE MATRIX
A single layer of flat, cuboidal and columnar cells that are in direct contact with the amniotic fluid. It is from this layer that amniotic MSC (AMSC) are isolated and stored to be used for regenerating tissues. There are no nerves, muscles, or lymphatics.	Very similar to the basement membrane found in the other parts of the body like the conjunctiva or gingiva. The basal lamina contains large amount of proteoglycans like heparan sulfate that is one of the major proteoglycan in the gingiva.	The amniotic mesoderm layer consists of macrophages and fibroblast-like mesenchymal cells. The spongy layer on the stromal portion of the amnion has an abundance of hydrated proteoglycans and glycoproteins that form a non fibrillar network along with collagen.

TABLE 3: VARIOUS SUBSTANCES FOUND IN DIFFERENT LAYERS OF AM[6]

LAYER	COMPONENTS SECRETED/PRESENT	FUNCTIONS
Epithelial cells	Collagen type III, IV Non collagenous glycoproteins: nidogen, fibronectin,vitronectin Laminin 5 TNF alpha, NGF, BDNF, Noggin, activin	Helps in cellular adhesion of gingival cells, invasive growth of fibroblasts and angiogenesis in the early phases of wound healing
Basement membrane	Integrin a6/b4 – main ligand	Construction of the hemidesmosome like structure that favours the adhesion and anchoring of ESCs to the healing wound. Facilitates migration of epithelial cells, reinforces adhesion of basal epithelial cells, promotes epithelial differentiation, and prevents apoptosis.
Connective tissue matrix	Mitogenic factors, antiangiogenic and anti-inflammatory proteins and natural inhibitors to proteases Growth factors like keratinocyte growth factor (KGF), basic-fibroblast growth factor (b-FGF), transforming growth factor-beta (TGF-β), nidogen growth factor (NGF), and epidermal derived growth factor (EDGF) Markers like CD90+, CD105+, CD73+, CD44+, HLA I+, CD45, CD34, CD11b	Provide a natural healing environment, has anti scarring property Promote periodontal regeneration Accelerate the inflammatory phase towards the proliferative phase that allows the wound to heal in a much faster and efficient way.

MECHANISM OF WOUND HEALING BY HUMAN AMNIOTIC MEMBRANE[6]

TABLE 4: MECHANISM OF WOUND HEALING BY AMNIOTIC MEMBRANE

	PROPERTIES	MECHANISM
1.	Immunomodulatory	unique molecular surface architecture and biochemical properties derived from the layer of trophoblast cells which renders it insusceptible to maternal immune attack Express low levels of major histocompatibility complex (MHC) class I surface antigens and reduced levels of the major components of the antigen processing machinery. Do not express MHC class II antigens, the costimulatory molecules CD80 (B7-1), CD86 (B7-2), CD40, or CD40 ligand, in the presence or absence of interferon gamma (IFN-γ), one of the most potent known inflammatory cytokines. Neither express the programmed cell death receptor 1 (PD1) (an inhibitory receptor that is normally expressed on activated T and B cells), nor its two ligands: programmed death ligands 1 and 2 (PD-L1 and PD-L2) and the immunoglobulin-like transcript receptors 2, 3, and 4 (ILT R-2, ILT R-3, and ILT R-4).
2.	Antimicrobial	Forms a firm adherence barrier with the wound via fibrin and elastin linkages that seals the wound and prevent contamination. mediated by two mechanisms: direct; via secretion of antimicrobial factors such as LL-37 and indirectly, via secretion of immunomodulative factors which will upregulates bacterial killing and phagocytosis by immune cells Many bactericidal products of purine metabolism and lysozyme are also found Defensins, mostly beta 3 defensins, secretary leukocyte proteinase inhibitor (SLPI) and elafin act as components of the innate immune system to provide protection from infection. Cystatin E, an analogue of cysteine proteinase inhibitors and a powerful antiviral is secreted in large amount by the amniotic cells.

3.	Reduction of pain	Diminishes inflammation and provides a better state of hydration that soothes the wound bed to promote faster healing Soft mucoid lining of amniotic membrane protects the exposed nerve endings from external irritant that help to decrease pain sensation.
4.	Anti scarring	Secretes vascular endothelial growth factor (VEGF), hepatocytes growth factor (HGF) that maintains a proper balance between TGF-1 and TGF-3 that prevents scarring. Down-regulates TGF-beta and its receptor expression by fibroblast that causes a reduced fibrosis at the site. This also modulates the healing of a wound by promoting tissue reconstruction. Various immune cells like T cell, dendritic cell and B cell are actively suppressed that prevents pathological remodeling and excessive fibrosis.
5.	Anti inflammatory	MSCs in the AM decrease the secretion of the proinflammatory cytokines like tumor necrotic factor-alpha (TNF-alpha) and interferon (IFN) Increase in the production of anti-inflammatory cytokines interleukin (IL) 10, IL -4, IL-1alpha and IL-1beta. HAE and HAM cells express various anti angiogenic and anti inflammatory proteins such as interleukin 1 receptor antagonist; tissue inhibitors of metalloproteinase (TIMPs) 1, 2, 3, 4 and IL-10. Reduces recruitment of various inflammatory cells including polymorphonuclear cells, CD3 cells, CD4 T cells, and CD11b cells to the injured site thereby reducing the inflammation.
6.	Revascularization	Release of angiogenic factor like insulin derived growth factor (IGF) that promotes granulation tissue formation and epithelialization.

ADVANTAGES[5]:
TABLE 5: ADVANTAGES

HUMAN PLACENTA	AMNIOTIC MEMBRANE CELLS
• Readily available • Normally discarded organ so fewer ethical concerns • Less environvental and age related DNA damage as it is a neonatal tissue • Non- invasive procurement procedure	• Abundant cells that are sufficient for cell replacement therapies • Possess immunomodulatory properties so reduced risk of rejection or reaction on transplantation • No tumorigenicity • Relatively easy isolation so cost effective • Possess stem cell like characteristics

APPLICATIONS:
The use of placental tissue for the treatment of wound started more than 100 years ago when by Davis in 1910 first used these fetal membranes as skin substitutes for the treatment of open wounds. Later these membranes were also used for the treatment of burn and repair of conjunctiva defects and as a dressing of chronic ulcers. In 1965, Dino et al. demonstrated for the first time that amniotic membrane could be separated, sterilized and safely used at a later date. Since then a lot of research has been initiated to understand the true regenerative potential of this membrane.[6]

The AM can be used either alone with only amniotic epithelium (intact AM) or without it (denuded AM); Cryopreserved, freeze dried or in hyperdried form. It can also be used in powder form in the areas of suture lines and hard to cover areas. These fetal membranes are being used as a graft or dressing in the management of burns; alternative treatment to manage wounds in the oral cavity like 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 like permeability, stability, elasticity, flexibility, plasticity, and resorbability also makes 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.[6] Antiinflammatory and antiscarring property of AM 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.[6] HAM has been tried in the reconstruction of TMJ ankylosis as it prevents fibrosis and reankylosis when used as an interpositional material.[8] AM is even used as a carrier for local delivery of the various drugs like antibiotic netilmycin (NTM) and antiviral drugs like acyclovir (ACV) and trifluridine (TFU).[9] Amnion has been tried as a graft material after vestibuloplasty where it prevents secondary contraction after the surgery and maintains the postoperative vestibular depth.[10]

AMNIOTIC MEMBRANE AND PERIODONTAL THERAPY: A LITERATURE REVIEW
Periodontal diseases leading to deterioration of tooth-supporting structures are a serious concern for clinicians. Spatially-directed regeneration of periodontal tissues through manipulation of cell fate pathways is referred to as guided tissue regeneration (GTR). The technique involves the use of a semipermeable membrane underneath the gingiva precluding downward regeneration of gingival epithelium along root surface while maintaining the space for regeneration of periodontal ligament and establishment of connective tissue attachment.[11] The clinical application of amniotic membrane for guided tissue regeneration while fulfilling the current mechanical concept of GTR, amends it with the modern concept of biological GTR.[12] Though the amniotic membrane has been used in general surgery for a long period of time, its use in dentistry particularly in periodontal surgeries is only new to us. Very few studies have been done so far. Holtzclaw DJ retrospectively reviewed the practice's electronic database which revealed that 114 patients were treated with GTR therapy from March 2010 to October 2011. Of these patients, 64 were treated with Amnion-Chorion membrane (ACM) combination GTR therapy and had ≥12 months of follow-up. All patients were diagnosed with localized moderate-to-severe chronic periodontitis and exhibited radiographic evidence of ≥1 vertical osseous defect. All patients were treated by thorough degranulation of intrabony periodontal defects and placement of bone allograft covered by ACM. Clinical measurements 12 months after surgery revealed an average probing depth reduction of 5.06 ± 1.37 mm and clinical attachment level improvement of 4.61 ± 1.29 mm.[13] Ghahroudi AA et al compare the efficacy of amnion allograft and connective tissue graft in covering denuded root surfaces. Seventy-one teeth in 22 patients with gingival recession were treated randomly with coronally displaced flap plus connective tissue graft (control group, n = 29 recessions in 10 patients) or coronally displaced flap plus amnion allograft. They concluded that amnion allograft might be a suitable alternative to connective tissue graft in procedures to cover denuded root surfaces and can reduce recession depth.[14] similarly; Shetty SS et al[15] and Gurinsky B[16] successfully obtained complete root coverage using amniotic membrane.

These membranes are extremely thin around 300 nm in cross

sectional thickness unlike the other collagen membranes used for guided tissue regeneration which are around 700 - 800 nm. Because of its thin diameter it intimately molds according to the defect anatomy and root surfaces easily. Though it is known to keeps its strength and morphology at least one month in vitro when soaked in sterilized physiologic saline solution at room temperature, further investigation is needed to evaluate its ability to resist the masticatory forces, biodegradable rate for subsequent repair and maturation of the mucosal tissues when used as a barrier membrane.[6]

Conclusion

The preserved human amniotic membrane is a novel tissue engineerined biomaterial that is recently tried in field of medicine and dentistry to regenerate the lost tissues and accelerate repair.[6] 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 post-operative scarring and subsequent loss of function and providing a rich source of stem cells.[12] However, further research and long term clinical trials investigating the full potential of this stem cell reservoir are still warranted to strengthen the fact amniotic membrane is indeed a reservoir for regeneration.[6]

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