
Amniotic Membranes - A Futuristic Trend in Periodontal Regeneration

NASHRA KAREEM

Senior lecturer, Department of Periodontics, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai, India

*Corresponding Author

Email: nashrak.sdc@saveetha.com

Abstract: One of the most frequent indications of periodontal plastic surgery is the treatment of gingival recessions. This treatment has mainly been justified by the patient's wish to improve the aesthetic appearance when there is an exposed root. The ultimate goal of a root coverage procedure is complete coverage of the recession defect with a good aesthetic appearance related to the adjacent soft tissues, minimal probing depth following healing and minimal postoperative discomfort. The selection of one surgical technique over another with the use of particular grafts depends on several factors to achieve optimal regeneration of the lost periodontal tissues via guided tissue regeneration (GTR). Over the years, various materials have been used as physical barriers in GTR. One such novel material that has been tried relatively recently is the amniotic or the placental membrane. The placental allografts possess various properties that may be beneficial as a barrier membrane in periodontal therapy. Therefore, this review article elaborately explains the basic features, actions and applications of amniotic membranes that can be used as a remarkable tool in the field of periodontal regeneration.

Key words: placental membrane, guided tissue regeneration, gingival recession, coronally-advanced flap.

INTRODUCTION

Regeneration is defined as "a reproduction or reconstitution of a lost or injured part. It is, therefore, the biologic process by which the architecture and function of lost tissues are completely restored." (Bosshardt and Sculean, 2009) This implies regeneration of the tooth's supporting tissues, including alveolar bone, periodontal ligament, and cementum.

It is a well known concept that the cells that repopulate the exposed root surface after periodontal surgery define the nature of the attachment that will form. Therefore, the major factor believed to prevent periodontal regeneration after conventional therapeutic approaches is the migration of epithelial cells into the defect area at a faster rate than that of mesenchymal cells, which leads to the formation of a long junctional epithelium and the prevention of the formation of a new attachment apparatus over the previously diseased root surface.

Gingival connective tissue cells can also populate the space adjacent to the denuded root surface after conventional periodontal treatment. Repopulation of the exposed root surface by gingival connective tissue cells is speculated to result in the formation of a connective tissue attachment followed by root resorption. Based on this speculation, the goal of regenerative procedures is to prevent apical migration of gingival epithelial and connective tissue cells and to provide maintenance of a wound space into which a selective population of cells (hence guided tissue regeneration [GTR]) is allowed to migrate, favoring the formation of a new periodontal attachment. Our team has rich experience in research and we have collaborated with numerous authors over various topics in the past decade (Deogade, Gupta and Ariga, 2018; Ezhilarasan, 2018; Ezhilarasan, Sokal and Najimi, 2018; Jeevanandan and Govindaraju, 2018; J *et al.*, 2018; Menon *et al.*, 2018; Prabakar *et al.*, 2018; Rajeshkumar *et al.*, 2018, 2019; Vishnu Prasad *et al.*, 2018; Wahab *et al.*, 2018; Dua *et al.*, 2019; Duraisamy *et al.*, 2019; Ezhilarasan, Apoorva and Ashok Vardhan, 2019; Gheena and Ezhilarasan, 2019; Malli Sureshbabu *et al.*, 2019; Mehta *et al.*, 2019; Panchal, Jeevanandan and Subramanian, 2019; Rajendran *et al.*, 2019; Ramakrishnan, Dhanalakshmi and Subramanian, 2019; Sharma *et al.*, 2019; Varghese, Ramesh and Veeraiyan, 2019; Gomathi *et al.*, 2020; Samuel, Acharya and Rao, 2020)

Biological Basis of Guided Tissue Regeneration

The biologic basis of guided tissue regeneration (GTR) is based on the assumption that the placement of physical barriers prevents apical migration of the epithelium and gingival connective tissue cells of the flap and provides a secluded space for the inward migration of periodontal ligament cells (PDL) and mesenchymal cells

on the exposed root surface, which in turn promote periodontal regeneration.(Gottlow, Nyman and Karring, 1984)

Besides favoring selective repopulation of the wound area, physical barriers are also thought to provide protection of the blood clot during the early phases of healing and to ensure space maintenance for ingrowth of a new periodontal apparatus. GTR membranes, as physical barriers, however, provide no biologic effects on differentiation and proliferation of mesenchymal and PDL cells, which is likely to limit their clinical efficacy.

Since the discovery that only selected cells have the potential to re-create a new periodontal attachment, a wide range of natural and synthetic materials such as methylcellulose acetate, expanded polytetrafluoroethylene (ePTFE), collagen, polyglycoside synthetic polymers and calcium sulfate, derived from a variety of sources, were tested for effectiveness and used as a physical barrier in GTR. Previously from my research facility, a lot of work has been done on plenty of topics in periodontology (Ramesh, Ravi and Kaarthikeyan, 2017; Ravi *et al.*, 2017; Arjunkumar, 2018; Gajendran, Parthasarathy and Tadepalli, 2018; Jain and Nazar, 2018; Kavarthapu and Thamaraiselvan, 2018; Ramesh *et al.*, 2018, 2019; Ezhilarasan, Apoorva and Ashok Vardhan, 2019; Kaarthikeyan, Jayakumar and Sivakumar, 2019; Kavarthapu and Malaiappan, 2019; Murthykumar, Arjunkumar and Jayaseelan, 2019; Vijayashree Priyadharsini, 2019). Therefore this review article elaborately explains the basic features, actions and applications of amniotic membranes that can be used as a remarkable tool in the field of periodontal regeneration.

Amniotic Membranes

One of the new materials which have been tried recently for guided tissue regeneration includes placental membranes. The placental allografts possess antibacterial and antimicrobial properties with immunoprivilege and are thus quite different from cadaveric allograft, xenograft and alloplast barrier membranes used in periodontal therapy.

Historical Background

Human amniotic membranes have been used successfully for a wide range of applications for over 70 years. The use of fetal membrane for skin transplantation was first reported by *Davis* in 1910.(Davis, 1910) Description of the use of human amniotic membrane for burned and ulcerated skin surfaces was given by *Stern* in 1913.(Stern, 1913) They evaluated the accelerative effect of the membrane on epithelialization and the reduction in pain by its application on burned or ulcerated sites. In 1940, *De Roth* first reported the use of fetal membranes in the ocular surface. He used fresh amnion and chorion as a biological dressing material for management of conjunctival defects.(de RÖTTH, 1940)

Interest in utilization of amniotic membrane waned in the early 1980's as a result of communicable diseases such as H.I.V/A.I.D.S, Hepatitis, etc. In the late 1990's and early 2000's amnion re-appeared in cryopreserved form for the treatment of ophthalmic wounds. There were only few reports in the literature on reconstruction of oral tissues using amnion. *Lawson* in 1985 studied the use of amniotic membrane along with pectoralis major muscle for oral cavity reconstruction.(Lawson, 1985) He concluded that placement of amnion over the deep aspect of the muscle that is exposed to the oral cavity resulted in a more rapid development of mucosa. When muscle was used without an amniotic membrane, the healing process usually took twice as long. Also, when amnion was not used, it showed a significant amount of wound contracture.

Anatomy of Amniotic Membranes

Amniotic membranes have their origin from extra-embryonic tissue. This tissue is composed of a fetal component (the chorionic plate) and a maternal component (the deciduas). The fetal component includes the amnion and chorion membranes which separate the fetus from the endometrium.

The structure of amniotic membrane has three parts which are the following (Figure 5);

- a. Epithelial monolayer
- b. Thick basement membrane
- c. Avascular stroma
- i. Compact layer
- ii. Fibroblast layer
- iii. Intermediate layer

Epithelial monolayer

Epithelial monolayer consists of a single layer of cells which are arranged uniformly on the basement membrane. It is the innermost layer, lies nearest to the fetus, and is also called the amniotic epithelium. The amniotic epithelial cells have an active secretory and transport function as suggested by their ultrastructure.(Sadler, 2006) The epithelium is firmly fixed to a basement membrane which is in turn attached to a condensed acellular layer. This layer is composed of collagen types I, II, and V. Blood vessels or nerves are absent in amniotic membranes. It derives its nutrition directly by diffusion out of the amniotic fluid.

Basement membrane

The basement membrane is quite remarkable as it is one of the thickest membranes found in all human tissues and provides support to the fetus throughout gestation. It is similar to that of conjunctiva in its distribution of collagen type IV subchains.(Niknejad *et al.*, 2008)

Avascular stroma

The main fibrous skeleton of amniotic membrane is formed by the compact layer of stroma lying adjacent to the basement membrane. Next layer, the fibroblastic layer containing mesenchymal cells is responsible for secretion of different types of collagens. Predominant types are interstitial collagens (types I and III) which form parallel bundles to maintain the mechanical integrity of the membrane. Filamentous connections between interstitial collagens and epithelial basement membranes are provided by collagens types V and VI. The last layer which is known as intermediate layer or spongy layer or zona spongiosa lies adjacent to the chorionic membrane and contains a meshwork of mostly type III collagen. Its spongy appearance is the result of the presence of abundant content of proteoglycans and glycoproteins.(Parry and Strauss, 1998)

The chorion consists of two layers: an outer formed by the primitive ectoderm or trophoblast, and an inner by the somatic mesoderm; with this latter, the amnion is in contact (Figure 6). The trophoblast is made up of an internal layer of cubical or prismatic cells, the cytotrophoblast or layer of Langerhans, and an external layer of richly nucleated protoplasm devoid of cell boundaries, the syncytiotrophoblast (Table 1).

Properties of Amniotic Membrane (Figure 7)

- a. **Lack of Immunogenicity:** Occurrence of acute rejection after transplantation of amniotic membranes is negated by the fact that amniotic epithelial cells do not express the following human leukocyte antigens (HLA) HLA-A, HLA-B, HLA-D, and HLA-DR antigens but express HLA-G, HLA-1, interferon γ and other immunologic factors on their surfaces. This implies that immunosuppressive properties of amniotic membrane can increase the chances of successful grafting.(Chopra and Thomas, 2013) As tissue grafts of placental membrane materials present a low risk of immune rejection, they are considered to be “immune privilege”(Sakuragawa, Tohyama and Yamamoto, 1995).
- b. **Antimicrobial and Antiviral Properties:** Amniotic membranes have the ability to produce β -defensins, the predominant type being $\beta 3$ -defensin. Antiviral properties are exhibited by the presence of cystatin E, the analogue of cysteine proteinase inhibitor. There is still further need for studies to verify these properties of the amniotic membrane. Amniotic membrane may prevent infiltration and adhesion of microorganisms to wound surfaces by acting as a barrier. The hemostatic property of collagen fibers of amniotic basement membrane prevents hematoma formation in clean surgical wounds. This reduces bacterial load and risk of infection by preventing accumulation of microbes.
- c. **Promotion of Epithelialization:** Amniotic membrane facilitates migration of epithelial cells, reinforces basal cell adhesion, promotes epithelial differentiation, prevents epithelial apoptosis and promotes epithelialization in healing of wounds by the production of various growth factors. Its basement membrane serves as a safe and suitable bed for the growth of epithelial cells. Sufficient oxygenation for epithelial cells is provided by its good permeability in contrast to other synthetic materials. Thus, amniotic membrane is an ideal tissue which facilitates the growth of epithelial cells, helping in their migration and differentiation.(Koizumi *et al.*, 2000)
- d. **Inhibition of Fibrosis:** The amniotic membrane possesses antifibrosis property. Fibroblasts are naturally responsible for scar formation during wound healing and are activated by transforming growth factor- β . Amniotic membrane reduces the risk of fibrosis by downregulation of transforming growth factor β and its receptor expression by fibroblasts. Therefore, scaffold of an amniotic membrane modulates wound healing by promoting reconstruction of tissues rather than promoting formation of scar tissue.(Choudhury, Nisha and Padmanabhan, no date; Lee *et al.*, 2000)
- e. **Biomechanical Properties:** Thickness of normal amniotic membrane lies between 0.02 and 0.5 millimetres which includes around 6–8 layers of cells. An average surface area of this membrane is about 1600 square centimetres. An important property of amniotic membrane is its resistance to various proteolytic factors owing to the presence of interstitial collagens. Elastin present in amnion is responsible for providing elasticity. It has multiple metabolic functions such as its role in water and soluble material transportation and production of bioactive peptides, growth factors, and cytokines.(Solomon *et al.*, 2001)
- f. **Inhibition of Inflammation and Angiogenesis:** It is hypothesized that it decreases influx of inflammatory cells to the wound area and consequently reduces inflammatory mediators by serving as a barrier. Matrix metalloproteinases released by infiltrating neutrophils and macrophages are removed by inhibitors of matrix metalloproteinases found in the amniotic membrane. The presence of proteinase inhibitors may facilitate wound healing. Thrombospondin-1, secreted by the amniotic epithelium, acts as an antiangiogenic factor.

Two very potent proinflammatory mediators, interleukin-1 α and interleukin-1 β , are suppressed by matrix of stroma of amniotic membrane.(Arai et al., 2012)

- g. Cell Differentiation Property: The fetal placental tissues have the potential to transform into different cell lineages. The hematopoietic lineage is found in the chorion, allantois, and yolk sac; and the mesenchymal lineage is found in both the chorion and amnion. The cells isolated from the chorion are good sources of cells of hematopoietic and mesenchymal lineages as they possess these properties. It is considered that the amniotic membrane can maintain pluripotent stem cell potential for cell differentiation.
- h. Reduction of pain - These membranes diminish inflammation and provide a better state of hydration that soothes the wound bed to promote faster healing. Soft mucous lining of amniotic membrane protects the exposed nerve endings from external irritant that help to decrease pain sensation.(Hori et al., 2006)

Preparation and Isolation Of Membrane

All donated tissue should follow a strict guideline for procurement, processing and distribution before they can be used clinically. Special processing and sterilization is recommended to ensure consistent quality and preservation of the properties of the placental membranes. Various methods have been tried which include:

- a. Hypothermic storage at 4°C
- b. Freeze drying through liquid nitrogen at -196°F
- c. γ -sterilization
- d. Glycerol preservation
- e. Cryopreservation

The media and storage temperature used for the preservation process affects the viability of cells and growth factors in the membranes.

Sterilization with γ -rays has no significant effect on growth factor content in the human placental membrane. While storage of these membranes in glycerol at 4°C will result in immediate cell death, cooling will preserve the membrane for an indefinite time and make it bacteriologically pure and immunologically inert.

Cryopreservation with dimethylsulphoxide (DMSO) at -80°C is an important modality for preservation of these tissues as it keeps them viable for a longer period of time but causes loss of some angiogenic factors and cell rupture.(Diwan and Stevens, 1976) To overcome these problems with cryopreservation, freeze dried - irradiated (Lyophilized) is the one the most commonly used preservation technique that preserves the original size and shape with minimum cell rupture. The membrane is first freeze dried at -60°C under vacuum (atmospheric pressure 102 mm of Hg) for 48 hours and then irradiated with 2.5 mega Rads (25 K Gray) in a batch type cobalt-60 irradiator.

The far-infrared rays and microwaves are also used for sterilization of the placental membrane which is known as the Hyper-dry-amnion. During the drying process, the temperature inside the hyperdrying device should not exceed 35°C as high temperatures on the surface that can reach 60°C can decrease the degradation of tissue-protein.

Compared to cryopreserved amnion, which can be preserved for less than 3 months at 80°C, “Hyper-dry amnion” can be preserved at room temperature indefinitely until the packet is cut open. It is easily cut to the desired size and shape just before application. The freeze dried membrane can be readied for use by soaking in normal saline for 1 minute. It returns to a layered structure similar to that of fresh amnion when it absorbs water unlike the hyperdry amniotic membrane.

Glutaraldehyde fixation is a recently introduced method to fix the placental membrane that provides better stability and properties. This requires neither antibiotics nor the use of special storage techniques and renders the amnion sterile and non-immunogenic. Glutaraldehyde treated amnion is employed successfully as a microvascular interpositional graft in many experimental animals and is the area of further research.(Thomson and Parks, 1984)

Even after proper sterilization by any technique, a proper screening to 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 form a mandatory step. Though amnion membrane is considered totally non immunogenic, low-grade inflammatory responses occur with viable amniotic epithelial cells. The immunogenicity of cryopreserved tissue is less than that of fresh tissue as more than 50% of amniotic epithelial cells remain viable for two months.

Clinical Applications of Amniotic Membrane

1. The physical properties of placental membranes have proven it to be compatible with corneal surfaces of the eye.
2. The use of human placental membrane as a surgical wound dressing in treatment of leg ulcers, skin loss in Stevens-Johnsons diseases, reconstruction of the pelvic floor, vaginal epithelialization, replacement of normal mucosa in Rendu-Osler-Weber diseases, and ear surgery has been described

3. The membranes can be used to manage wounds in the oral cavity like that of tongue, buccal mucosa, vestibule, palatal mucosa, and floor of the mouth.
4. The use of human placental membrane for overlying epithelial defects after flap necrosis following surgery in the head and neck region have been reported with good results.
5. Placental membrane can be used as an interpositional material for the reconstruction of TMJ ankylosis as it has anti-fibrotic properties and thus can prevent re-ankylosis.
6. Owing to its antimicrobial properties, placental membrane can be even used as a carrier for local delivery of the various drugs.

Application of Amniotic Membranes in Periodontal Regeneration

1. It may be used as a graft material in lower ridge vestibuloplasty.
2. These membranes can be used to line the floors of cortical bone defects and to cover the superficial surface of these defects.
3. They may be used for the treatment of shallow to moderate Miller's class I and class II recession defects.
4. Amniotic membranes are also used to treat class II furcation defects.
5. They can be used for ridge preservation following tooth extraction.
6. They may be used for regeneration in the treatment of periodontal intrabony defects in localized, moderate to severe chronic periodontitis cases.

Our institution is passionate about high quality evidence based research and has excelled in various fields ((Pc, Marimuthu and Devadoss, 2018; Ramesh *et al.*, 2018; Vijayashree Priyadharsini, Smiline Girija and Paramasivam, 2018; Ezhilarasan, Apoorva and Ashok Vardhan, 2019; Ramadurai *et al.*, 2019; Sridharan *et al.*, 2019; Vijayashree Priyadharsini, 2019; Chandrasekar *et al.*, 2020; Mathew *et al.*, 2020; R *et al.*, 2020; Samuel, 2021)

Concluding Remarks

The clinical application of placental membranes 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. However, further research and long term clinical trials with larger sample sizes are required to thoroughly investigate the complete potential of amniotic membranes as a tool in periodontal regeneration.

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CONFLICT OF INTEREST

The author declares that there is no conflict of interest.

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Table 1: Substances Found In Different Layers Of Amniotic Membranes

LAYER	COMPONENTS PRESENT/SECRETED	FUNCTIONS
Epithelial cells	Collagen type III, IV Non collagenous glycoproteins : nidogen, fibronectin, vitronectin Laminin-5, tumor necrosis factor(TNF) α , nerve growth factor (NGF), brain derived neurotrophic factor (BDNF) , Noggin, activin	Helps in cellular adhesion of gingival cells, invasive growth of fibroblasts and angiogenesis in the early phases of wound healing. Favours the adhesion and anchoring of embryonic stem cells (ESCs) to the healing wound.
Basement membrane	Integrin $\alpha6\beta4$ – main ligand	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), nerve 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.

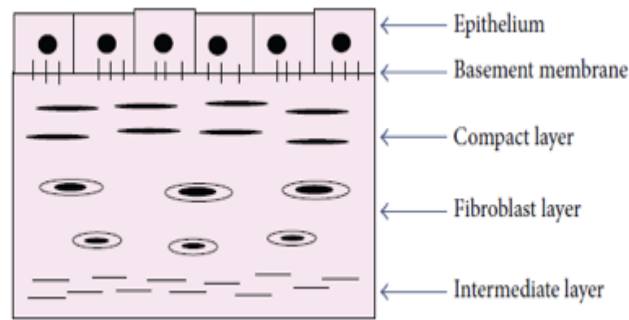


Fig.1: Line diagrammatic representation of histological architecture of amnion

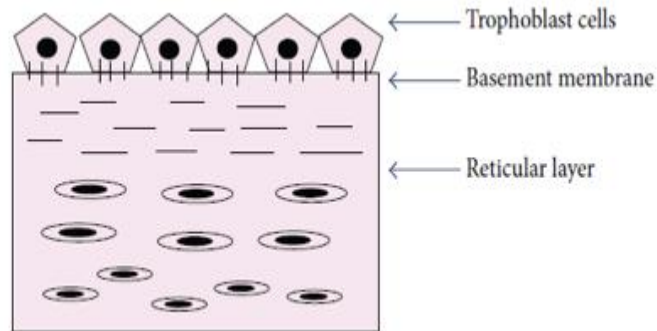


Fig.2: Line diagrammatic representation of histological architecture of chorion

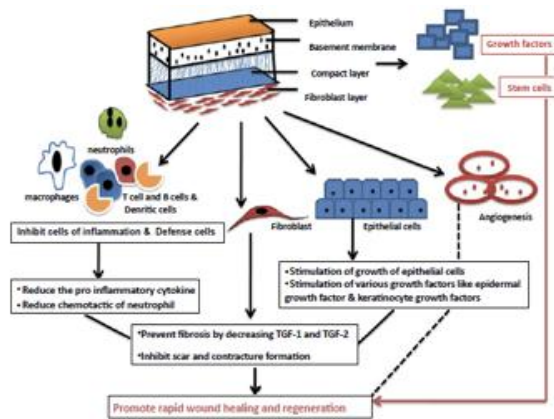


Fig.7: Mechanism of action of amniotic membrane