

## Placental tissues: fixing smiles

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**ABSTRACT:** Autograft tissue currently remains the gold standard of periodontal plastic surgery. It provides excellent predictability, improved long-term outcomes, and superior aesthetics over other treatment options. The amniotic sac encloses the developing foetus through gestation and is composed of amnion and chorion tissues. Amnion basement membrane closely mimics the basement membrane of human oral mucosa. Use of placental allografts in dentistry is a more recent development. The currently available dental form of placental allograft is composed of cryopreserved, dehydrated amnion chorion laminate. Fresh amnion has generally been used. When compared to traditional guided tissue regeneration membranes, placental barriers, such as amnion chorion membranes demonstrate many unique properties including anti-adhesive effects, bacteriostatic properties, wound protection, pain reduction, and epithelialization effects.

**KEYWORDS:** Amniotic membrane, Allograft, Placental membrane.

### INTRODUCTION

The human placenta is a complex organ which starts developing within few days after fertilization and is very important for development and survival of the fetus throughout the gestation. It is about 10-15 micrometer thick which constitutes of two fetal membranes, the inner amniotic membrane and the outer chorion.<sup>1</sup>

The amnion membrane encases the amniotic fluid and fetus, and is highly flexible because of which it is easily separated from the chorion.<sup>2</sup>

Amniotic membrane is the innermost lining of fetal membrane that is in contact with the developing fetus. The lining serves as a natural barrier to protect the fetus from infections and trauma because of lack of immune system.<sup>3</sup>

Amnion is thin, tough, transparent membrane. Chorion side of the membrane is rougher and porous. Amnion can easily be separated from chorion leave and placenta as far as the umbilical cord. Once separated, the amnion is found to be smooth and shining and much tougher and more elastic and easier to clean than the thicker chorion, which does not strip from the placenta. The chorion, although thicker, is more easily torn because it is much less elastic.<sup>4</sup>

### HISTORY

Davis introduced the use of human fetal membranes for skin transplantation in 1910 and 3 years later Sabella described its use for burnt and ulcerated skin surfaces. The use of amniotic membranes offers the advantage that the thicker intact membrane can be handled easily and remains on the wound longer than amnion by itself. In 1952, Douglas reported the use of amniotic membrane to temporarily cover burn wounds. Since then this material has shown good results as a temporary wound dressing and is mostly used to treat burns.<sup>5</sup>

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.<sup>6</sup>

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. 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 amniotic membrane, the healing process usually took twice as long. Also, when amnion was not used, it showed a significant amount of wound contracture.<sup>5</sup>

#### **PROPERTIES OF AMNIOTIC MEMBRANE**

The human amnion membrane is a biological graft which has unique properties like anti-adhesive effects, bacteriostatic properties, wound protection, pain reduction and epithelialisation effects.<sup>4</sup>

#### **IMMUNOMODULATIVE AND IMMUNE PRIVILEGE**

Amniotic membrane has a unique molecular surface architecture and biochemical properties that is derived from the layer of trophoblast cells which renders it unsusceptible to maternal immune attack. The native amniotic epithelial cells express the non-polymorphic, non-classical human leukocyte antigen (HLA-G) but lack the polymorphic antigens HLA-A, B (Class IA) and HLA-DR (Class II) on their surfaces. The class I antigen is seen in almost all cells of the amniotic membrane unlike the class II antigen which is only present in some fibroblasts. These mesenchymal stem cells are different from other nucleated mammalian cells as stimulate they show little allogeneic reactivity when administered to MHC unmatched adult immune competent recipients. This immune barrier is due to lack of expression of co-stimulatory cell surface molecules such as CD80 and CD8 mostly in the human. Furthermore, they are actively suppressive of T cell, dendritic cell and B cell function that down-modulate exuberant inflammation.<sup>1</sup>

#### **ANTI-MICROBIAL PROPERTY**

It forms an early physiologic "seal" with the host tissue precluding bacterial contamination. It forms a firm adherence barrier with the wound via fibrin and elastin linkages that seals the wound and prevent contamination. This tight adherence helps in restoring lymphatic integrity, protects circulating phagocytes from exposure and allows faster removal of surface debris and bacteria from the wound.<sup>1</sup>

This antimicrobial activity of mesenchymal stem cells is mediated by two mechanisms: direct; via secretion of antimicrobial factors such as LL-37<sup>6</sup> and indirectly, via secretion of immunomodulative factors which will upregulates bacterial killing and phagocytosis by immune cells<sup>7</sup>. These mechanisms will reduce the bacterial counts in the wound and promotes healing. Many bactericidal products of purine metabolism and lysozyme are also found in the amnion membrane. Defensins, mostly  $\beta$ -3 defensins that helps the epithelial surfaces to resist microbial colonization form a major group of anti-microbial peptides found in the amniotic membranes.<sup>1</sup>

#### **REDUCTION OF PAIN**

Amnion membrane has the unique ability to reduce the pain during the surgical procedure as it diminishes inflammation and provides a better state of hydration that soothes the wound bed to promote faster healing. The soft mucoid lining of amniotic membrane also protects the exposed nerve endings from external irritant that help to decrease pain sensation by preventing nerve stimuli. It also supports the growth of the epithelium and facilitates migration and reinforced adhesion.<sup>1</sup>

#### **ANTI SCARRING AND ANTI INFLAMMATORY PROPERTIES**

Wound healing stimulates recruitment of various cells like neutrophils, macrophages and giant cells to the surgical site to combat the invading microorganisms and to control the ongoing inflammatory process. Macrophages and giant cells at the surgical site produce cytokines that attract fibroblasts leading to development of fibrosis at the surgical site. Fibroblasts are activated by the transforming growth factor (T.G.F.) beta that is secreted by macrophages and fibroblast in the wound area. Amniotic membrane secretes vascular endothelial

growth factor (V.E.G.F.), hepatocytes growth factor (H.G.F.) that maintain a proper balance between TGF-1 and TGF-3 that prevents scarring.

The mesenchymal cells in amniotic membrane decreases the secretion of the proinflammatory cytokines like TNF- $\alpha$  and interferon while simultaneously increasing the production of anti-inflammatory cytokines IL-10, IL-4, IL-1 $\alpha$ , IL-1 $\beta$ .<sup>1</sup>

#### **INCREASED VASCULARIZATION AND REVASCULARIZATION**

There is an enhanced induction of Vascular Endothelial Growth Factor (V.E.G.F.) both for V.E.G.F. receptors 1 and 2 by the cells of the amniotic membrane. Extensive neovascularization observed immediately after its application is attributed to the release of angiogenic factor like insulin derived growth factor (I.G.F.) that promote granulation tissue formation and epithelialization.<sup>1</sup>

#### **INCREASED EXTRACELLULAR MATRIX DEPOSITION**

Mesenchymal cells differentiation helps in regenerating the damaged tissue, whereas mesenchymal cells paracrine signaling regulates the local cellular responses to injury. The paracrine signaling by the mesenchymal cells help in cell survival, proliferation, migration and gene expression of epithelial cells, endothelial cells, keratinocytes and fibroblasts.<sup>8</sup>

#### **ANTI-ADHESIVE**

Human amniotic membranes preserved at -80<sup>0</sup>C for one month revealed the presence of E.C.F., T.G.F.- $\alpha$ , K.G.F., H.G.F., b-F.G.F., T.G.F.- $\beta$ <sub>1</sub>, T.G.F.- $\beta$ <sub>2</sub>. The basement membrane facilitates migration of epithelial cells, reinforces adhesion of basal epithelial cells and may promote epithelial differentiation.<sup>9</sup>

#### **SOURCE OF AMNIOTIC TISSUE**

Eligible amnion donors are living mothers that have delivered a live baby through cesarean section or vaginal section.<sup>4</sup>

An elective caesarean delivery helps in the right choice of a consenting donor and planned collection of amniotic membrane because placenta collected after natural vaginal delivery may have structural defects linked with stretching of the membrane during labour and delivery and may be infected by normal vaginal flora, herpes, chlamydia or other contaminant bacteria. Due the risk of infection with H.I.V. and hepatitis C, tissue transplantation laws in different countries require different protocols for preservation, testing and storage.<sup>10</sup>

Protection against transmission of viruses is effected by donor selection and testing for serological markers of presently known transmissible viruses at the time of donation and again 3-4 months later. This time window omits any chances of infection transfer that may be diagnosed later on.<sup>4</sup>

#### **PROCESSING OF AMNIOTIC MEMBRANE**

For clinical use of the membrane, it can be prepared in the following forms:

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

#### **FRESH MEMBRANE**

Studies have shown that amnion can be maintained in viable condition for up to 6 weeks if stored aseptically at -48<sup>0</sup>C in 0.5% silver nitrate solution or in 20% glycerine solution or in sterile saline after passage through one rinse of 0.025% sodium hypochlorite solution.<sup>11</sup>

Preservation in 85% glycerol is not only very simple, it is also suitable for preservation over longer periods (up to 5 years). Glycerol dehydrates tissue by physically replacing most of the intracellular water but does not change the cell's ionic concentration, thus it is an efficient agent that preserves tissue by protecting cell integrity.<sup>12</sup>

Immediately prior to their use, small clean sections ( $6 \times 10 \text{ cm}^2$ ) of membrane were cut and kept in 400 ml of saline containing 10,00,000 IU penicillin at  $48^\circ\text{C}$  up to 24 hours.<sup>11</sup>

### **DRIED MEMBRANE**

After cleaning and rinsing the membranes are spreaded on plastic sheet and allowed to dry in open air. These are found to be equally effective when compared with fresh.<sup>4</sup>

### **FROZEN MEMBRANE**

Hypothermic storage at  $4^\circ\text{C}$ , freeze drying through liquid nitrogen at  $-196^\circ\text{F}$ , cooling preserve the membrane for an indefinite time, produces bacteriologically pure and immunologically inert material.<sup>4</sup>

### **FREEZE DRIED IRRADIATED**

Membrane after obtaining from placenta dried at  $-60^\circ\text{C}$  under vacuum for 48 hrs. It is then irradiated with 2.5 mega rads in a batch type cobalt -60 irradiator. By the method of freeze drying there is sublimation of liquid moisture to gaseous state without having undergone the intermediate solid state. This method helps the membrane to maintain its original size and shape with minimum cell rupture. The freeze dried membrane can be made ready for use by soaking it in normal saline for 1 minute.<sup>4</sup>

### **CRYOPRESERVED**

Cryopreservation with dimethylsulphoxide at  $-80^\circ\text{C}$  is an important modality for preservation of these as it keeps the membrane viable for a longer period of time but causes loss of some angiogenic factors and cell rupture.<sup>13</sup>

### **STABILIZED AMNIOTIC MEMBRANE**

Successful use of gluteraldehyde treated amnion is employed as a microvascular interpositional graft in experiment animals.<sup>4</sup>

### **HYPER-DRIED AMNIOTIC MEMBRANE**

The far Infrared and microwaves are used for sterilization of amniotic membrane which is known as hyper-dry-amnion. The temperature during drying should not exceed  $35^\circ\text{C}$ . Hyper-dried amnion can be preserved at room temperature until packet is cut open.<sup>1</sup>

### **MEMBRANE PLACEMENT**

Membrane is applied with rough (chorionic) surface next to the wound. Cryopreserved amniotic membrane is manufactured with the stromal side of the graft attached to the nitrocellulose filter paper that is sticky unlike the epithelial side which is non-sticky and shiny. Fibrin glue sticks to the stromal side and permits adherence of the membrane to the wound even without suturing.<sup>1,4</sup>

### **USE OF AMNIOTIC MEMBRANE**

Periodontal diseases leading to deterioration of tooth supporting structure are a serious concern for clinicians. The clinical application of amniotic membrane for guided tissue regeneration while fulfilling the current mechanical concept of guided tissue regeneration, amends it with the modern concept of biological guided tissue regeneration. Biomechanical guided tissue regeneration proposed here in using amnion membrane, not only maintains the structural and anatomical configuration of regenerated tissues, but also contribute to the enhancement of healing.<sup>14</sup>

When compared to traditional guided tissue regeneration membrane, placental barriers demonstrate many unique properties that have been described earlier.<sup>15</sup>

Human amniotic membrane has been tried in temporo mandibular joint ankylosis as it prevents fibrosis and reankylosis when used as an interpositional material.<sup>1</sup>

Amnion has been used as a graft material after vestibuloplasty where it prevents secondary contraction after the surgery and maintains the post-operative vestibular depth.<sup>16</sup>

Hyper dried amnion or cryopreserved amniotic membrane tissue is used as a barrier membrane in the treatment of periodontal osseous defect with or without bone graft<sup>15</sup> and even tried in the management of gingival recession with guided tissue regeneration.<sup>17</sup>

#### LIMITATIONS

- Operator's inexperience is a limitation.<sup>15</sup>
- The amniotic membrane is a biological-derived material and concomitant are the same problems of other biological material applications. For instance, transmission of infectious diseases is always a risk associated with human organ and tissue transplantation. Thus, the same precautions and safety criteria applied to organ transplantation have to be adhered in the application of amniotic membrane. Potential donors need to be screened effectively for any risk factors that might render them unsuitable for donation. A review of relevant medical records to ensure freedom from risk factors for and clinical evidence of H.I.V., hepatitis B, hepatitis C, C.M.V., syphilis, and other possible infections, should be carried out. There is a slight possibility that the donor may be in the "window period" of infection. Hence, even if serological tests are negative, it is advisable to repeat the investigations after 6 months. The amniotic membrane can be preserved at -80°C until samples found negative of any infectious diseases.<sup>18</sup>
- Cryopreserved / Hyper-dried membranes are costlier.<sup>3</sup>
- Membrane is highly fragile.<sup>4</sup>

#### CONCLUSION

The use of amniotic membrane over the past 100 years has produced a significant amount of use and success in multiple areas of medicine and now-a-days its use has increased in dentistry too due to its unique properties. Human amniotic membrane is a uniquely suited material for use as an allograft. Used in its natural form, then later in preserved preparations, the material assists in the healing process through a number of physical, biochemical and molecular biological pathways to promote regenerative healing while simultaneously reducing scar formation. Additional research and characterization of this process will more completely define the dramatic results seen in the application of this material.

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