



## USE OF AMNIOTIC MEMBRANE AS A DRESSING IN ORAL AND MAXILLOFACIAL REGION- A REVIEW

### KEYWORDS

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### ABSTRACT

*The need to cover the epithelial defect has meant that a number of materials including mucosal and skin grafts are regarded as suitable for grafting in oral and maxillofacial surgery. To bypass the disadvantages of other materials such as skin, biological membranes have been suggested as options, including fetal amniotic membrane. The amniotic membrane has been considered a suitable tissue for allograft based on its low immunogenicity. It also possesses anti-inflammatory, anti-fibrotic, and anti-angiogenic properties and also accelerates wound healing and epithelialization. Human Amniotic Membrane serves as a basement membrane that facilitates epithelial cell migration, reinforces adhesion of basal epithelial cells and prevents epithelial apoptosis.*

*This article is an attempt to review the spectrum of Human Amniotic Membrane as a dressing in Oral and Maxillofacial region. We also report a case of Cervical Necrotizing Fasciitis, treated with the help of the membrane and achieving primary closure with satisfactory post-operative results.*

### INTRODUCTION

Wound healing is a complex and intricate mechanism involving numerous cellular and extracellular events culminating in the production of a mature scar. Thus, formed scar could be a tissue indigenous to the site or a replacement. A common problem encountered by a surgeon is a non-healing wound because of local or systemic influences or both. Although the wound healing process occurs in an orderly fashion, the outcome of a wound healing is influenced by biologic principles of wound care, debridement, irrigation, topical and systemic medicaments and dressings.

An epithelial defect not responding favorably to routine modalities of dressings instigated the search of an ideal dressing material which is biologic and satisfies certain desirable properties. The ideal dressing should provide relief from pain, protect wound from secondary infection, maintain a moist environment, prevent loss of fluids, promote healing, be elastic and non-antigenic, adhere well to wound, be easy to apply, easily available and economical.<sup>1</sup>

The search for such a material led researchers to human amniotic membrane which augurs well with expectations. Human amniotic membrane is a biologic dressing that is non immunogenic because of its unique characteristic of not expressing major histocompatibility antigens. Hence it does not evoke an immune response from the host. In addition to that, the membrane produces various growth factors including basic fibroblast growth factor, transforming growth factors, angiogenic growth factors, all contributing to better epithelialization.<sup>2,3</sup>

This article is an attempt to review the use of amniotic membrane dressing in various oral and maxillofacial defects, along with a case report of amniotic membrane dressing in cervical necrotizing fasciitis.

### HUMAN AMNIOTIC MEMBRANE

The Amniotic Membrane (AM) is considered a bio-therapeutic product composed of a single layer of epithelial cells that lie on a basement membrane and of a nonvascular collagenous stroma.<sup>4</sup>The

properties for a dressing satisfied by human amniotic membrane are unique in their anti-adhesive effect, bacteriostatic property, wound protection, pain reduction and epithelialization.<sup>5</sup> Because of its anti-adhesive effect, the amnion becomes cohesive to the wound and then peels off by itself on epithelialization. This probably happens because amnion is believed to induce arrest in tissue proliferation.<sup>5</sup> It acts as a protective barrier under which healthy healing at the wound site can occur. This was proven in the use of the membrane in oral defects.<sup>5</sup> Wound contracture is also reduced by the use of AM, as evidenced by the study done by Lawson.<sup>6</sup> AMs, as revised by Atiyeh et al, preserve a healthy excised wound bed and maintain a low bacterial count in contaminated wounds, decrease loss of protein, electrolytes, fluids, and energy, decrease risk of infections, avoid bulky dressings, minimize pain, and accelerate epithelial regeneration, thus shortening hospital stay.<sup>7</sup> Ilancheran et al discussed stem cell-like properties, differentiation potential, and immunologic and anti-inflammatory properties of cells isolated from fetal membranes.<sup>8</sup>

Embryologically, amniogenesis begins during transformation of morula to blastocyst stage i.e. 7-8 days after fertilization. There is separation of the inner cell mass of germ disc at the periphery of ectodermal cells to form a slit like cavity. With appearance of extra embryonic mesoderm this layer separates from primitive trophoblast. Majority of the new cells are derived from preexisting epithelium. They become flattened as pregnancy progresses towards term.<sup>9</sup> By second month of gestation mesenchymal cells separate from epithelium by a layer of tissue containing moderate amounts of loosely packed collagen fibrils. Degenerative changes occur in the mesenchymal fibroblasts during the 5<sup>th</sup> and 6<sup>th</sup> month of gestation. At term, fetal membrane consists of a single layer of ectoderm derived amnion cells firmly attached to the collagen rich mesenchyme of 6-8 cell thickness. The interphase between the two can be easily separated by finger dissection.<sup>9</sup>

The AMs harvested from placentas of selected and screened donors are invariably contaminated. Washings with antibiotic solutions, lyophilization, sterilization with cobalt-60 radiation, and glycerol preservation have been used to free AMs from bacterial and fungal

contamination.<sup>7</sup> Although irradiation is the most suitable method for sterilization of tissue allografts, very few studies have reported on the clinical effectiveness of radiation-sterilized AM.<sup>10</sup> Glycerol-preserved AMs have been used with great success in burn injuries.<sup>10</sup> Singh et al reported no significant differences in clinical parameters evaluated for radiation-sterilized AMs compared with glycerol-preserved AMs.<sup>10</sup>

#### **AMNIOTIC MEMBRANE AS A DRESSING**

The first documented use of Amniotic membrane was reported in skin transplant in the year 1910.<sup>11</sup> In 1913, studies reported use of amniotic membrane on burnt and ulcerated surfaces. The study observed lack of infection in clean sterile wounds and an increased rate of epithelialization.<sup>12,13</sup> Successful use in epithelialization of conjunctival defects was reported by an ophthalmologist in 1940.<sup>14</sup> The use of amniotic membrane as "amnioplastin" in the prevention of meningo-cerebral adhesions following head injury was reported. The membrane was treated in 70 % alcohol and then dried in oven. Microscopic examination revealed continual Dural repair with no organized adhesions or evidence of adhesion or rejection.<sup>15</sup>

Experiments on relationship of fetal membranes and bacterial growth in burns proved the membrane to equal to isograft and superior to allograft or xenograft. The study also reported marked reduction in pain and immediate adherence of the membrane as dressing. They showed it was very effective in partial thickness wounds.<sup>16</sup> Another study showed that when the mesenchymal surface was placed against full thickness wounds with precautions to prevent drying of the membrane; there was reduced pain, fluid loss and 50 % reduction in infection.<sup>17</sup>

The membrane was successfully used in non-healing wound in diabetics, as a graft over surgical defect of glossectomy.<sup>18</sup> A study using pectoralis major muscle flap with amnion for oral cavity reconstruction indicated high rate of primary take than one would anticipate.<sup>6</sup>

An otolaryngology study concluded that low antigenicity, high antimicrobial potential and the ability to foster epithelialization makes amniotic membrane excellent in covering surfaces after flap necrosis.<sup>19</sup> Another study concluded that AM transplantation may be considered an alternative method for treating persistent epithelial defects and sterile ulceration that are refractory to conventional treatment by conjunctival flaps or tarsorrhaphy.<sup>20</sup> It has also been shown in a study that AM with a limbal autograft can be a promising surgical treatment for reconstructing ocular surface in patients with recurrent pterygium associated with symblepharon.<sup>21</sup>

A study on the use of amnion as graft material for vestibuloplasty concludes it to be a potential graft material for vestibuloplasty procedures.<sup>5</sup> In another study done in 2011, Kothari et al evaluated the use of AM as a grafting material for vestibuloplasty procedures. They concluded that grafts of AM remain viable and reliable for covering of raw surface, prevent secondary contraction after vestibuloplasty and maintain the postoperative vestibular depth.<sup>22</sup>

In a study by Tuncel and Ozgenel, they evaluated the use of AM as an interpositional material to prevent temporomandibular joint reankylosis in randomized rabbit model. They concluded that interpositional arthroplasty with AM was superior to gap arthroplasty.<sup>23</sup> Another study by Tuncel et al in rabbit models concluded that interpositional arthroplasty with AM and temporalis muscle fascia graft have an almost similar effect in preventing ankylosis of Temporomandibular joint.<sup>24</sup>

A histological study on gingival wound healing with transplanted human amniotic membrane indicates induction of rapid epithelialization and both granulation tissue and collagen formation but suppression of inflammation with rapid gingival wound healing.<sup>25</sup>

In a study done by Kesting et al, they evaluated the use of human AM as a grafting material for midpalatal oronasal fistulas in seven Berlin minipigs. The study showed successful closure of the fistulas with the AM and offers promise as a simple tension free closure for such fistulas.<sup>26</sup> In another study done on rats by Tsuno et al, they concluded that hyperdry AM is a suitable new dressing material in cleft palate repair.<sup>27</sup>

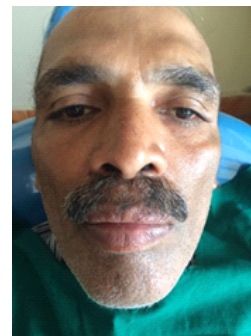
A study by Lima et al on rats have showed that AM can be used as a biologic dressing in oral mucositis following 5-fluorouracil administration. It goes on to show the potential AM possesses in managing mucositis after chemo-radiation therapy.<sup>28</sup> In another study by Kar et al, they evaluated the clinical outcome of surgical repair of oral mucosal defects using cryopreserved human AM as a graft material. Results indicated that AM promotes healing and epithelialization without specific complications.<sup>29</sup>

As stated above, the Amniotic membrane has got a widespread application in the field of oral and maxillofacial surgery and its use will increase manifolds in the days to come.

Following is a case of Cervical Necrotizing Fasciitis, where the AM was used for dressing of large surgical wound that could not be closed primarily.

#### **REPORT OF A CASE**

A 50-year-old male patient was referred to our department with complaints of painful swelling in the chin area of 11 days duration. Patient is a known case of diabetes mellitus and under medication since four years. He gave a history of swelling and pus discharge from right lower third molar region, for which he visited a local hospital. He was advised to undergo the extraction of the right lower third molar along with incision and drainage. As planned, incision and drainage was done 8 days back, followed by the extraction of the offending tooth the next day. After the planned treatment, patient was put on a course of antibiotics (Amoxicillin and Metronidazole) and analgesics. The patient reported of no improvement in his condition and pus discharge started again from the chin and the third molar region since two days.



**Figure 1- Preoperative**

On clinical examination, a diffuse swelling was noted in the submental area with local rise in temperature. There was discharge of pus mixed with blood from the site, with presence of an erythematous area. Overlying skin appeared to be necrotic, associated with crepitus. No tenderness was elicited on palpation. On intraoral examination, bone was found to be exposed in the area of right lower third molar region, where extraction was done. The localized gingiva was soft and edematous, with rolled out margins. Systemically, the patient was afebrile and showed no signs of stress. Fasting blood Sugar and Post Prandial blood Sugar levels were 116.2 mg/dl and 199.3 mg/dl respectively.

Orthopantomogram (OPG) was advised for radiological examination, which reveals altered bony trabeculae in relation to right lower second molar. A significant radiolucent depression was seen in the

area of right lower third molar region.

A diagnosis of Cervical Necrotizing Fasciitis secondary to Osteomyelitis with respect to right lower third molar was made, based on clinical and radiological correlation.



**Figure 2- Necrosed portion of neck**



**Figure 3- Post debridement**



**Figure 4- Placement of Amniotic membrane**

Immediate empirical antibiotic therapy consisted of penicillin, gentamycin, and metronidazole administered intravenously. Pus was sent for culture and sensitivity. It showed numerous pus cells and presence of rare gram negative bacilli, which showed no growth after 36 hours of incubation. Initial surgical plan was Wound debridement and primary closure. Necrosed skin and underlying fat and fascia were excised. Surgical debridement resulted in a large skin defect with slough that could not be closed primarily and required good wound care with frequent dressings. Based on its proved safety and clinical efficacy in treatment of non-healing ulcers of different etiologies, Amniotic Membrane was chosen as a temporary wound dressing.

Amniotic Membrane was used, following the protocol described by Manjunath et al.<sup>30</sup> The AM was prepared from placenta harvested during cesarean section. Eligible mothers were screened based on medical history and physical examination to decrease the risk of disease transmission. Donors were also tested seronegative for human immunodeficiency virus, hepatitis B and C. Under sterile

condition, the amnion was separated from the chorion by blunt dissection and washed 4 times in sterile normal saline followed by 0.05% sodium hypochlorite solution and 4 washings in distilled water. It was then stored for a minimum of 12 hours with saline solution containing gentamicin 0.40 mg/mL. The AM was then refrigerated for 5 days at 4°C in sterile bottles containing 85% glycerol solution before it was used on the patient. Until the AM was ready to be used on the patient, regular wound packing with wet-to-dry gauze soaked in saline, dilute hydrogen peroxide, and povidone iodine was continued 3 times daily.

Once ready, the AM was applied to cover the entire defect after thorough cleaning and debridement of the wound under sterile conditions. Subsequently, the AM was secured over the wound with sterile gauze pads and roller bandages. Similar dressings were repeated every alternate day. The dressings were continued for fourteen days. The entire wound healed almost completely in 2 weeks. No primary closure was required at the later date. At the end of 6-month follow-up there was no significant cosmetic and functional deformity. There was no line of demarcation between the involved skin and adjacent skin not involved in the infection.

### **CONCLUSION**

The AM when used as a temporary dressing over non healing wounds increases the rate of healing and granulation tissue formation, making the recipient defect more apt. AM is easy to preserve, non immunogenic, economical, easily available, easy to apply and has minimum dressing requirements after application. Glycerol preserved amnion could be used as an ideal dressings for ulcers, burns and Cervical Necrotising Fasciitis. A developing nation requires such exemplary techniques to manage the exorbitant number of wounds encountered on a daily basis. The idea of "amniotic membrane banking" at every hospital could be an answer.

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