

## Amniotic Membrane a Novel Material for Root Coverage: A Case Report

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### ABSTRACT

**Background:** Periodontal plastic surgical procedures aimed at coverage of exposed root surface. As procuring of sufficient graft from donor site may be difficult for the treatment of root coverage procedures, so various alternative additive membranes have been used. Several treatment modalities have been applied over the years to achieve periodontal regeneration with no predictable outcome. However, in the field of medicine, mesenchymal stem cells derived from human foetal membrane have shown promising results. Amniotic membrane not only maintains the structural and anatomical configuration of regenerated tissues but also contributes to enhancement of healing. The aim of the case report is to evaluate the efficacy of the processed dehydrated allograft amnion in treatment of Millers class II gingival defects.

**Materials and method:** Root coverage with dehydrated allograft with coronally advanced flap in the treatment of gingival recession.

**Result:** At 3 months after surgery, there was an average increase of 2mm of new gingival tissue defect coverage.

**Conclusion:** Based on the data collected from case report, processed dehydrated allograft amnion may provide an effective alternative to autograft tissue in treatment of gingival recession.

**Keywords:** Periodontal Plastic surgery, Amniotic membrane, Root coverage, Allograft.

### INTRODUCTION

Periodontal disease is a chronic inflammatory condition that occurs in response to predominantly gram-negative bacterial infection originating from dental plaque<sup>[1]</sup>.

Prior to 1950s, periodontitis was treated mostly by tooth exfoliation or extraction, and that is still the predominant treatment for most of the world's population today. Until the 1980's, the most commonly used treatment consisted of scaling and root planning, followed by resective surgery aimed at achieving zero pocket depth. During the 1980s, data were obtained demonstrating that the thoroughness of root debridement and subgingival

infection control, not the presence or absence or periodontal pockets, is the major determinant of successful periodontal therapy, and non-surgical therapy became a commonly used treatment. Neither resective surgery nor non-surgical therapy results in significant regeneration of periodontal attachment. Recent data clearly show that regeneration of the previously destroyed periodontal attachment tissues is biologically possible, and regeneration has become the goal of therapy for the 1990s<sup>[2]</sup>.

Regeneration by grafting may be further enhanced by the use of barrier membranes that exclude

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gingival fibroblasts and epithelium from the healing site. Still further enhancement seems to be possible by local application of various growth factors although studies in this important area are now only in their infancy. The future of periodontal therapy is exceedingly bright [3]. However, the current regenerative procedures have limitations in attaining complete and predictable regeneration, especially in advanced periodontal defects [4].

For successful periodontal regeneration, formation of a functional epithelial seal, insertion of new connective tissue fibers into the root, reformation of a new acellular cementum on the tooth surface and restoration of alveolar bone height are required. The complex events associated with periodontal regeneration involve recruitment of locally-derived progenitor cells that can differentiate into periodontal ligament cells, mineral-forming cementoblasts, or bone-forming osteoblasts [5, 6].

Applications of amnion membrane include chemical or thermal burns, correction of corneal epithelial defects, neurotrophic corneal ulcers, leaking blebs after glaucoma surgery, reconstruction of conjunctival and ocular surfaces, ocular cicatricial pemphigoid or Stevens-Johnson syndrome, and bullous keratopathy. These membranes have also been used in furcation defects, intrabony defects, and gingival recession coverage [7].

Gingival recession is the term for the exposure of root surface due to apical migration of gingival margins. Many patients seek treatment because of concerns about aesthetic appearance, root sensitivity, or fear of early loss of the affected teeth. However, other complications can also arise, such as root caries and tooth discolouration [8]. The goal of treating gingival recession is to restore the gingival margin to the cemento-enamel junction (CEJ) and create normal sulcus with a functional attachment [9].

Today, the concept of excluding the gingival epithelium with the mere use of barrier membranes has evolved to the incorporation of the correct signalling molecules like the growth factors and the presence of correct cell population like the stem cells, fibroblast, and cementoblast directly into the wound to promote regeneration. Mesenchymal stem cells (MSCs) are one of the major cells population that play an important role in mediating

each phase of the wound healing process: Inflammatory, proliferative, and remodelling.

Recently, the foetal derived MSCs from the placenta or other gestational tissues like the amniotic fluid, umbilical cord are novel materials with rich stem cell reserves. The use of placental tissue for the treatment of wound started > 100 years ago when Davis in 1910 first used these foetal membranes as skin substitutes for the treatment of open wounds [10].

Dino *et al.* [11] demonstrated for the 1<sup>st</sup> time that amniotic membrane could be separated, sterilized and safely used at a later date. Amnion-derived cells with multipotent differentiation ability have attracted lot of attention in the regeneration of periodontal tissues. Amnion lines the innermost portion of the amniotic sac of the placenta. Its structure consists of a single layer of epithelium cells, thin reticular fibers (basement membrane), a thick compact layer, and a fibroblast layer. The basement membrane contains collagen type III, IV, and V and cell-adhesion bioactive factors including fibronectin and laminins [12]. Data suggest the amnion basement membrane closely mimics the basement membrane of human oral mucosa [13].

## MATERIALS AND METHOD

### PREPARATION OF AMNIOTIC MEMBRANE

In the production of amnion allograft which was used in this study, consent was taken from mothers regarding the use of amnion and associated tissues during caesarean section surgery. All donated tissue follows strict guidelines for procurement, processing, and distribution, as dictated by the Tissue Bank, (Tata Memorial Hospital, Mumbai). These safety measures include testing for serological infectious diseases such as human immunodeficiency virus (HIV) type 1 and 2 antibodies, human T-lymphotropic virus 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. Upon collection of the maternal tissue, the amnion and chorion tissues are carefully separated, and the amnion is cleansed prior to processing. The

allograft is terminally sterilized, dehydrated, perforated, and terminally sterilized.

## PATIENT SELECTION

Systemically healthy patient, non smoker with millers class II gingival recession was selected.

## PRESURGICAL TREATMENT

The patients were educated and motivated with an emphasis on proper oral hygiene maintenance. All the patients underwent the initial phase of treatment, which consisted of thorough scaling and root planing.

## SURGICAL PROCEDURES:-

At first 60 second pre-operative (Fig. 1) rinse with 0.12% chlorhexidine mouthwash was done and the surgical area was prepared with adequate anesthesia using 2% lignocaine hydrochloride containing 1:80,000 adrenaline. After that measurement of gingival defect

(Fig 2) was done from the cementoenamel junction to apical extent of the gingival margin in the recession defect. Preparation of exposed root surfaces which involved minimal flattening with hand instrumentation was done (Fig 3). 2-minute application of tetracycline solution (Fig 6) followed by saline rinse was done. Intracuticular incisions at the buccal margin of treated tooth and extending to the adjacent tooth to include the papilla with horizontal incisions made right angles to the adjacent interdental papillae, at the level of the CEJ. Two oblique vertical incisions ( Fig 4) along the adjacent teeth were extended beyond the mucogingival junction and a trapezoidal mucoperiosteal flap was raised ( Fig 5) to the point of MGJ. Full thickness flap was raised allowing for coronal positioning of the flap. De-epithelialization of adjacent papilla was done. The processed dehydrated allograft measurement was taken (Fig 8) was placed onto the exposed root surface . Upon placement, the processed dehydrated amnion allograft (Fig 9) becomes hydrated and self adheres to the exposed root. Immediately after placing the membrane, the reflected flap was coronal positioned over the dehydrated amnion allograft and secured with an interrupted suture technique ( Fig 10) . Care was taken not to move the allograft

after placement and during flap closure. Periodontal pack was given ( Fig 11).



Fig 1: pre-operative site.



Fig 2: Recession management.



Fig 3: SRP done in preoperative area.





Fig 4: vertical incision given.



Fig 7: Amniotic membrane.



Fig 5: Flap reflection done.

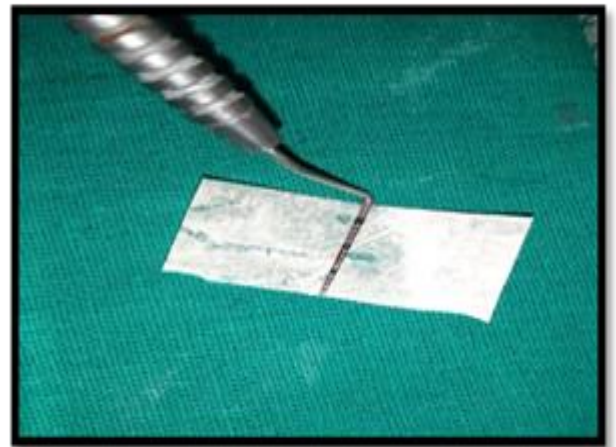


Fig 8: Measurement of amniotic membrane.



Fig 6: Tetracycline application done.



Fig 9: Amniotic membrane placed.



Fig 10: Flap advanced and sutured.



Fig 11: Periodontal pack given.



Fig 12: Preoperative photograph.

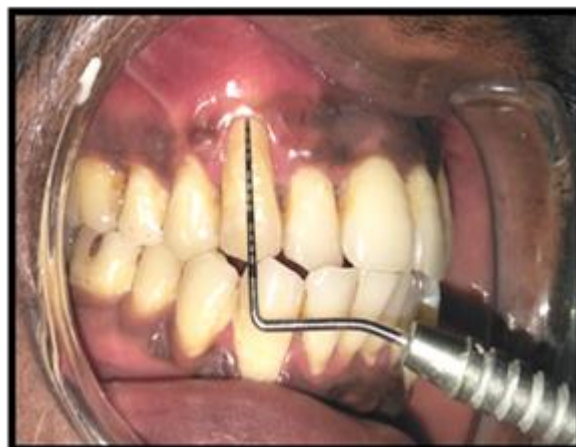


Fig 13: Post-operative photograph.

## RESULTS

At the end of 6 months significant difference was found from baseline ( Fig- 12) to 6 months as there was reduction in recession depth, recession width and gain in keratinized gingival. Post-operative results at 6months showed a decrease in recession depth from 4mm to 2mm (Fig- 13) and keratinized gingival increases from 0.70 mm to 1mm post operatively after 6 months.

Table 1: Data regarding recession height and keratinized tissue.

|             | RECESSION<br>HEIGHT | KERATINIZED<br>TISSUE |
|-------------|---------------------|-----------------------|
| BASELINE    | 4mm                 | 0.70mm                |
| 6<br>MONTHS | 2mm                 | 1mm                   |

## DISCUSSION

The ultimate goal of periodontal plastic surgical procedure for recession coverage is the complete regeneration of the supporting components of the periodontium, resulting in complete coverage of the denuded root surfaces in an esthetic as well as a functional manner. Several studies have shown the effectiveness and predictability of GTR for root coverage<sup>14,15</sup>.

Histologically, new bone and cementum with inserting fibers have been shown to form after recession coverage by GTR<sup>16</sup>. Amniotic membrane as a GTR membrane consists of a single layer of

epithelium cells, thin reticular fibers (basement membrane), a thick compact layer, and a fibroblast layer. The basement membrane contains collagen type III, IV, and V and cell-adhesion bioactive factors including fibronectin and laminins. Of particular interest is the fact that this amnion layer possesses several types of laminins, with laminin-5 being the most prevalent. It plays a role in the cellular adhesion of gingival cells and concentrations of this glycoprotein in amniotic allograft may be useful for periodontal grafting procedures<sup>17</sup>. Amnion tissue contains growth factors that may aid in the formation of granulation tissue by stimulating fibroblast growth and neovascularization. In addition, the cells found within tissue exhibit characteristics associated with stem cells and may enhance clinical outcomes<sup>18</sup>. Amnion has shown an ability to form an early physiologic "seal" with the host tissue precluding bacterial contamination<sup>19</sup> and multiple studies support amnion's ability to decrease the host immunologic response via mechanisms such as localized suppression of polymorphonuclear cell migration<sup>20</sup>.

## CONCLUSION

Human amniotic membrane is a uniquely suited material for use as an allograft in wound management and is rising in various fields of tissue engineering, medicine, regeneration biology, and stem cell research. 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. To conclude, amnion from discarded placenta can be an interesting source of cells for regenerative medicine. However, further research and long-term clinical trials are required for exploring the full potential of this stem cell reservoir.

## CONFLICTS OF INTEREST

The authors declare they have no potential conflict of interests regarding this article.

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