

Amniotic Membrane in Periodontics - A Research Study

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Abstract

Background: Periodontal diseases leading to deterioration of tooth-supporting structures are a serious concern for clinicians. The clinical application of amniotic membrane for guided tissue regeneration (GTR) while fulfilling the current mechanical concept of GTR amends it with the modern concept of biological GTR. Amniotic membrane not only maintains the structural and anatomical configuration of regenerated tissues but also contributes to the enhancement of healing.

Aim: The aim of our study was to evaluate the effectiveness of amniotic membrane in the treatment of various types of recession defects.

Materials and Methods: The sample size for the study was six cases, which were Miller's Class I or Class II gingival recession. Recession depth, recession width, keratinized gingiva, tissue width, and clinical attachment level (CAL) were recorded at baseline, 3, and 6 months postoperatively.

Results: 6 months after the root coverage procedures, the mean root coverage was found to be $72.3\% \pm 4.7\%$. CAL significantly decreased from 5.9 ± 0.62 mm preoperatively to 3.2 ± 0.7 mm postoperatively at 6 months, while keratinized gingiva showed significant improvement from 2.8 ± 0.26 mm preoperatively to 6.1 ± 0.58 mm postoperatively at 6 months.

Conclusion: 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.

Key words: Amniotic membrane, Guided tissue regeneration, Recession

INTRODUCTION

Periodontal disease is a chronic inflammatory condition that occurs in response to predominantly Gram-negative bacterial infection originating from dental plaque.¹

Before the 1950s, periodontitis was treated mostly by tooth exfoliation or extraction and that is still the predominant treatment for most of the world's populations today. Until

the 1980s, 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 of 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.²

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

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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.¹

However, current regenerative procedures have limitations in attaining complete and predictable regeneration, especially in advanced periodontal defects.³

For successful periodontal regeneration, the 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.^{4,5}

Advances in stem cell biology and regenerative medicine have presented opportunities for tissue engineering and gene-based approaches in periodontal therapy.^{6,7} These new approaches offer interesting alternatives to existing therapies for the repair and regeneration of the periodontium.

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.⁸

Periodontal plastic surgical procedures aimed at coverage of exposed root surface. Owing to the second surgical donor site and difficulty in procuring a sufficient graft for the treatment of root coverage procedures, various alternative additive membranes have been used. A recent resorbable amniotic membrane not only maintains the structural and anatomical configuration of regenerated tissues but also enhances gingival wound healing and provides a rich source of stem cells. Therefore, amniotic membrane is the choice of material these days in augmenting the better results in various periodontal procedures.

Diño *et al.*⁹ demonstrated for the first time that amniotic membrane could be separated, sterilized, and safely used at a later date. Amnion-derived cells with multipotent differentiation ability have attracted a 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, 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.¹⁰ Data suggest that the amnion basement membrane closely mimics the basement membrane of human oral mucosa.¹¹

Despite the introduction of allograft dermis tissue products and biologic mediators, autograft tissue remains the “gold standard” of periodontal plastic surgery as it provides excellent predictability, improved long-term root coverage, and superior esthetics over other treatment options.¹² Despite these clinical outcomes, the use of autograft tissue has drawbacks. Autogenous graft tissue is limited in supply, and its procurement significantly increases patient morbidity while also lengthening the duration of surgery.¹³

The utilization of amniotic membrane diminished in the early 1980's because of increase in the communicable diseases such as H.I.V and hepatitis. Amnion reappeared in the cryopreserved form for the treatment of ophthalmic wounds in the late 1990's and early 2000's.⁴ Lawson in 1985 was the first who 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. Furthermore, when amnion was not used, it showed a significant amount of wound contracture.¹⁴

Amnion Structure and Function

The amniotic membrane encases the amniotic fluid and fetus and is highly flexible because of which it is easily separated from the chorion.¹ Amniotic membrane has two types of cells - epithelial cells derived from embryonic ectoderm and amnion mesenchymal cells from embryonic mesoderm. At ultrastructural level, it is a thin, transparent, avascular composite membrane composed of three major layers, which is a single epithelial layer, a thick basement membrane, and an avascular mesenchyme consisting mainly of collagen, respectively. Amniotic membrane has no blood vessels or nerves; instead, the nutrients it requires are supplied directly by diffusion out of the amniotic fluid and/or from the underlining decidua. The amniotic epithelial cell (AEC) layer is 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 mesenchymal stem cells are isolated and stored for further regenerative use.¹⁵

Extracellular Matrix

Extracellular matrix materials form the structural components of the architecture of the membrane

and contain a variety of specialized proteins including fibronectin, proteoglycans, glycosaminoglycan, laminin, and other similar materials. The basal lamina contains a large amount of proteoglycans such as heparin sulfate that is one of the major proteoglycans in the gingiva. 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.¹⁶

The matrix of human amniotic membrane contains abundant growth factors such as keratinocyte growth factor, basic fibroblast growth factor, transforming growth factor-beta (TGF- β), nidogen growth factor, and epidermal-derived growth factor which promote periodontal regeneration. These growth factors provide a natural healing environment, accelerate healing, and mimic the stem cell niche for *ex vivo* growth.¹⁷

Benefits

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. The basement membrane of amniotic 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.⁸

Anti-inflammatory

The mesenchymal stem cells in the amniotic membrane decrease the secretion of proinflammatory cytokines such as tumor necrosis factor alpha and interferon while increasing the production of anti-inflammatory cytokines interleukin-10 and interleukin-4.¹ The pro-inflammatory mediators, interleukin-1 α and interleukin-1 β , are also suppressed by the matrix of stroma of amniotic membrane. The inhibitors of matrix metalloproteinases (MMPs) found in the amniotic membrane decreases MMPs released by infiltrating neutrophils and macrophages.^{1,8} Various tissue inhibitors of metalloproteinases 1, 2, 3, and 4, interleukin-10, and interleukin-1 receptor antagonists and endostatin which inhibit endothelial cell proliferation, angiogenesis, and tumor growth are also expressed by human amniotic epithelial and mesenchymal cells.⁸ It also reduces the recruitment of various other inflammatory cells including polymorphonuclear cells, CD3 cells, CD4 T cells, and CD11b cells to the injured site, thereby reducing the inflammation.^{1,8}

Antiviral and antimicrobial

Amniotic membrane firmly adheres with the wound through fibrin and elastin linkages that seal the wound and prevent contamination.¹ 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.¹⁸ Its antiviral properties are exhibited by the presence of a powerful antiviral agent, cystatin E which is an analog of cysteine proteinase inhibitors.^{8,15}

Anti scarring

Amniotic membrane secretes vascular endothelial growth factor (VEGF) and hepatocyte growth factor that maintains a proper balance between TGF-1 and TGF-3 that prevents scarring.¹

Angiogenesis

The cells of the amniotic membrane enhance the production of VEGF by activating the VEGF receptors 1 and 2. Extensive neovascularization after the application of amniotic membrane is due to the release of angiogenic factor such as insulin-derived growth factor that promotes granulation tissue formation and epithelialization.¹⁵

Immunomodulatory

The unique molecular surface architecture and biochemical properties of amniotic membrane that is derived from the layer of trophoblast cells renders it unsusceptible to maternal immune attack.¹ The native AECs express the non-polymorphic, non-classical human leukocyte antigen (HLA-G) but lack the polymorphic antigens HLA-A, B (Class IA) and HLA-D related (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 they show little allogenic reactivity when administered to major histocompatibility complex-unmatched adult immune competent recipients.¹⁵

Processing of Amniotic Membrane

For clinical use, amniotic membrane can be prepared in the following forms:¹

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

Amniotic Membrane in Dentistry

The use of amniotic membrane has recently increased clinically as an allograft material for chronic and acute

wound care management, for scar tissue reduction, as a barrier membrane, and as a soft tissue regeneration graft.¹⁹ The graft of amniotic membrane is a viable and reliable method to cover the exposed periosteum as they serve as a good alternative to mucosal and skin grafts.²⁰ Amnion allograft might be a suitable alternative to connective tissue graft in procedures to cover denuded root surfaces and can reduce recession depth.^{21,22}

It is easily available and preserved and is a cost-effective material.²³

Limitations of Amniotic Membrane

The use of amniotic membranes requires immense skill; thus, doctor's inexperience is a limitation. There is always an associated risk of infection transmissions. Amniotic membranes are fragile membranes, so they need to be dealt with very carefully. Cryopreserved membranes are expensive. The procedure associated with the use of these membranes is technique-sensitive and also depends on morphology of the defect.⁸

MATERIALS AND METHODS

Procurement of Amniotic Membrane

The procurement of the amniotic membrane was done by the Tissue Bank (Tata Memorial Hospital, Mumbai).

Subject Selection

Six subjects with the Miller's Class II gingival recession, who visited the Department of Periodontology, Indira Gandhi Government Dental College, Jammu, were included in the study. These patients were explained about the surgical procedure in detail and were included for the study with their consent. Maxillary canine site was selected for all the cases. All the patients were subjected to thorough scaling and root planning and were educated to maintain proper oral hygiene.

Clinical Evaluation

All the subjects were clinically evaluated by a single clinician trained for the specific purpose to measure the respective clinical parameters.

Recession depth, recession width, clinical attachment level (CAL), and width of keratinized gingiva at baseline, 3, and 6 months postoperatively for isolated recession on maxillary canine. Reference point for CAL was taken from cemento-enamel junction (CEJ) up to the base of the gingival sulcus. Width of the keratinized gingiva was measured from the margin of the gingiva up to the mucogingival junction.

Surgical Procedure

Double papilla flap technique was executed by giving two horizontal incisions at the CEJ followed by the vertical incisions placed at the line angles. The releasing incision was extended into alveolar mucosa. A partial thickness pedicle flap was raised by internal bevel incision, and the interdental papilla was undermined and separated from the underlying connective tissue.

Amniotic membrane was placed on the denuded root, and flap was sutured. Subjects were advised not to brush on the operated site for 21 days, and 0.2% chlorhexidine rinse was prescribed along with antibiotics and analgesics postoperatively. The patient was examined at 1st and 4th weeks to assess healing and then followed up at 3 and 6 months.

RESULTS

Post-operative results at 6 months show a mean decrease in recession depth from 3.75 ± 0.35 mm to 0.65 ± 0.25 mm. The recession width showed a mean decrease from 5.58 ± 0.52 mm to 1.9 ± 0.2 mm. The CAL showed mean decrease from 6 ± 0.9 mm to 3.65 ± 0.45 mm. The width of the keratinized gingiva showed a mean increase from 3.2 ± 0.3 mm to 6.3 ± 0.3 mm (Tables 1 and 2).

DISCUSSION

Gurinsky¹³ concluded an average increase of 3.2 mm (± 1.71) of new gingival tissue representing 97% (± 0.5) defect coverage in gingival recession with amnion allograft. Processed dehydrated allograft amnion demonstrated excellent esthetic results in terms of texture and color match. There were no adverse reactions during the course of this study, and patients reported relatively little post-operative discomfort. The ability of processed dehydrated allograft amnion to self-adhere eliminates the need for sutures, making the procedure less technically demanding and significantly decreasing surgical time.

Table 1: Pre-operative clinical evaluation

Subjects	Recession depth (mm)	Recession width (mm)	CAL (mm)	Width of keratinized gingiva (mm)
1	3.9	6.1	6.9	3.5
2	4	5.6	6	3
3	3.2	5.3	6.1	3
4	3.5	5	4	3.2
5	4.1	5.6	6.7	3.5
6	3.8	5.9	6.3	3

CAL: Clinical attachment level

Table 2: Post-operative clinical evaluation - 3 months

Subjects	Recession depth (mm)		Recession width (mm)		CAL (mm)		Width of keratinized gingiva (mm)	
	At 3 months	At 6 months	At 3 months	At 6 months	At 3 months	At 6 months	At 3 months	At 6 months
1	1.2	0.9	2.2	2.1	4.2	4.1	5.8	6.1
2	0.5	0.3	2	1.9	2.5	2.3	6.4	6.5
3	1	0.8	2	1.7	4	3.8	6.3	6.6
4	0.7	0.5	2.1	2	4.3	4.1	5.3	5.8
5	1	0.7	2	1.8	3.9	3.7	6.4	6.5
6	0.9	0.7	2	1.9	4	3.9	6.1	6.3

CAL: Clinical attachment level

Shetty *et al.*²⁴ compared bilateral multiple recession coverage with platelet-rich fibrin (PRF) to amniotic membrane. They concluded that the clinical outcome of the surgical procedure accounted for 100% root coverage, an enhanced gingival biotype, with both the membranes. The results were stable even after 7 months in the amniotic membrane-treated site. Thus, the use of amniotic membrane as a novel approach to root coverage is more advantageous than PRF due to the laboratory preparation of the autologous biomaterial and the use of the amniotic membrane as an additive material alternate to subepithelial connective tissue in reducing the need for a second surgical site is better advocated.

Velez *et al.*²⁵ concluded that the use of cryopreserved amniotic membrane provides significant cicatrization and wound healing after dental implant surgery. CAM supports the growth of epithelium, thus facilitating migration and reinforcing adhesion.

In accordance with the results of this study, amniotic membrane can be considered as a reliable alternative to autogenous connective tissue graft in the treatment of gingival recession as it avoids multiple surgeries in comparison to the latter.

CONCLUSION

Human amniotic membrane is a uniquely suited material for the 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.

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