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RESEARCH ARTICLE

NOVEL AMNION ALLOGRAFT IN THE TREATMENT OF GINGIVAL RECESSION COMPARED WITH CORONALLY ADVANCED FLAP

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ABSTRACT

Aims: To demonstrate the viability of amniotic membrane for the treatment of mucogingival defects (shallow-to-moderate Gingival recession defects).
Methods: The aim of this study was to record the use of processed dehydrated allograft amnion in the treatment of 20 shallow-to moderate recession defects defined as ≤3 mm of recession. Patients were randomly divided into 2 groups to receive either Amniotic membrane graft with coronally advanced flap Group-1 or Coronally advanced flap Group-2 and followed up for a period of 6 months.
Results: The results were statistically analysed using unpaired t test. The mean recession depth at baseline in both the groups was 2.75mm±0.78mm. 24 weeks postoperatively the mean recession depth was 0.15mm±0.36mm and group 2 it was 0.35mm±0.48mm. The difference between group 1 and 2 was not statistically significant in any of the occasions as analysed with independent samples t test. However, better root coverage was achieved in group 1 when compared to group 2 at 24 weeks postoperatively as compared with values at baseline.
Conclusion: Based on the data collected in this study, processed dehydrated amnion may provide an effective alternative to autograft tissue in the treatment of shallow-to-moderate Miller Class I and II recession defects.

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INTRODUCTION

The exposure of root surfaces due to gingival recession is a common occurrence. The exposed root surfaces pose a multitude of problems like hypersensitivity, root surface caries along with a massive esthetic deficiency. Covering exposed root surfaces has become an integral part of surgical periodontal practice. Indications for root coverage procedures include esthetic concerns of patients, root sensitivity, root caries, and lack of keratinized gingiva. Various surgical approaches and materials have been employed for the treatment of gingival recession. (Grupe, 1956; Nabers, 1966; Cohen and Ross, 1968; Bernimoulin *et al*., 1975; Langer and Lange, 1985; Raetzke, 1985; Tarnow *et al*., 1986) In most of these approaches, the exposed root surface is cleansed of bacterial endotoxins and regenerative material is placed over the defect. The materials used in these procedures include autogenous free gingival grafts, (Miller, 1985) autogenous connective tissue grafts, (Langer and Langer, 1985) and allograft dermis tissue. (Harris, 1998) Additionally, biologic mediators such as enamel matrix derivative, platelet-rich plasma, and recombinant platelet derived growth factor have been introduced into surgical protocols with the intent of accelerating and directing the wound healing. (Hagewald *et al*., 2002; Huang *et al*., 2005; Nevins, 2006) 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. (Harris, 2004) 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. Considering these pitfalls, many patients have an aversion to periodontal plastic procedures and delay or completely forgo treatment. Ultimately, this may cause the condition to worsen and possibly decrease the probability of successful outcomes when

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eventually treated. For these reasons, the dental profession has continued to search for an alternative soft tissue graft material that is effective and easy to use. Recently, new member in the category which is a suitable material for the mechanical modern concept of biological GTR is amniotic membrane which is freeze dried irradiated has been used as a substitute for the palatal donor sites to increase the width of keratinized tissue around teeth and implants, for the treatment of alveolar ridge deformities and for root coverage procedures. Processing of the dermis obtained from human donors removes all cells, thereby removing the source of disease transmission and immunologic reaction, leaving a structurally intact connective tissue matrix composed of type I collagen. The collagen matrix functions as a scaffold to allow in growth by host tissues. Thus the integrity of the extracellular matrix is maintained which is otherwise responsible for inflammatory response associated with the earlier available freeze dried amnion allograft. Amniotic membrane is widely used in both medicine and dentistry for reconstructive surgery. Originally developed to treat burn patients, it is now used in general, orthopedic and urogenital surgery in addition to its applications in dental surgical procedures. Since its introduction in 1999, there have been more grafts placed with no confirmed incidence of disease transmission. These unique characteristics make the Amnion Allograft completely biocompatible and safe. Hence the aim of this clinical study was to demonstrate the viability of amnion allograft for the treatment of mucogingival defects, the aim of this study was to record the use of processed dehydrated allograft amnion in the treatment of shallow-to-moderate gingival recession defects.

MATERIALS AND METHODS

This clinical study was carried out in the Department of Periodontics, Coorg Institute of Dental Sciences, Virajpet. The aim of this study was to record the use of processed dehydrated allograft amnion in the treatment of 20 shallow-to moderate recession defects defined as ≤3 mm of recession when there was no attached gingiva and or a lack of keratinized tissue around the defect and ≥ 3 mm of recession when the defect was surrounded by healthy tissue. Patients were randomly divided into 2 groups to receive either Amniotic membrane graft with coronally advanced flap Group-1 or Coronally advanced flap Group-2 by flipping a coin (F.F.S) immediately before surgery and followed up for a period of 6 months. Approval from the ethical committee of Coorg Institute of Dental Sciences was obtained. The nature and purpose of the study and the surgical protocol was explained to the subjects and a written consent was obtained before commencing the study.

Criteria for selection of Patients

Inclusion Criteria

1) Healthy individuals without any systemic diseases.
2) Patients who can follow the instructions and maintain a good oral hygiene.
3) Presence of two or more sites with Millers Class I recession in the anterior sextant.

Exclusion Criteria

1) Pregnant woman.
2) Patients undergoing orthodontic treatment.
3) Areas with severe malaligned teeth.
4) Patients who have undergone periodontal surgery in the last 6 months
5) Patients with any habit of tobacco usage.
6) Teeth in traumatic occlusion.

All patients were treated using the following surgical protocol:

1) 60-second preoperative rinse with 0.12% Chlorhexidine and local administration of 2% lignocaine with adrenaline, 1:80,000.
2) Measurement of gingival recession defect (RD**)** which is the vertical distance from cementoenamel junction to the marginal gingival was done using UNC-15 periodontal probe by a single investigator (Figure 1). The values were recorded at baseline, 1, 12 and 24 weeks.
3) Preparation of exposed root surfaces which involved minimal flattening with hand instrumentation.
4) Intrasulcular incisions at the buccal margin of treated tooth and extending to the adjacent tooth to include the papillae with horizontal incisions made at right angles to the adjacent interdental papillae, at the level of the CEJ. Two oblique vertical incisions along the adjacent teeth were extended beyond the mucogingival junction (MGJ) and a trapezoidal mucoperiosteal flap was raised to the point of the MGJ (Figure 2).
5) Split thickness dissection allowing for coronal positioning of the flap (Figure 3).
6) De-epithelialization of adjacent papilla.
7) The processed dehydrated allograft was placed onto the exposed root surface and proximal bone with the embossed side facing outward away from the tooth (Figure 4). Upon placement, the processed dehydrated amnion allograft becomes hydrated and self-adheres to the exposed root and proximal bone, thus eliminating the need for suture techniques (Figure 4).
8) Immediately after placing the membrane, the reflected flap was coronal positioned over the processed dehydrated amnion allograft and secured with either an interrupted or sling suture technique (Figure 5). Care was taken not to move the allograft after placement and during flap closure. A periodontal dressing was applied to the surgical site, post operative antibiotic and analgesics were prescribed. Patients were recalled after 1 week for reevaluation (Figure 6). Immediately following surgery, use of ice packs was recommended for 3 hours. All patients were instructed to discontinue tooth brushing, avoid trauma around the surgical site. A 0.2% chlorhexidine digluconate mouthwash rinse was prescribed 2 times (60 seconds) daily for the first 10 days. At 12 weeks, grafted defect sites were re-measured with a standard periodontal probe from the CEJ to the apical extent of the gingival margin (Figure 7). Gingivoplasty was not utilized in any case to smooth newly formed tissue. The same surgical protocol was followed for Group B without the placement of the graft.

Three weeks after surgery, the patients were instructed to resume mechanical tooth cleaning of the treated areas using a soft toothbrush and a roll technique. The patients were recalled for collection of data at 1,12and 24 weeks. On each visit, the area was checked for meticulous plaque control but no sub gingival instrumentation was performed, Clinical measurements were recorded both for the control and test sites.

RESULTS

A total of 40 labial gingival recession sites in 20 patients were considered for the study. The values obtained were tabulated and statistically analysed using unpaired t test. All statistical methods were carried out through SPSS for windows (version 17.0).

Recession depth

Comparison between values at the baseline and treatment end point in Group 1 and 2:

- The mean recession depth at baseline in both the groups was 2.75mm ±0.76mm.
- 12 weeks postoperatively, the mean recession depth in group 1 was 0.25mm±0.44mm and which was less when compared to group 2 with mean of 0.35mm±0.48mm.
- 24 weeks postoperatively the mean recession depth was 0.15mm±0.36mm and group 2 it was 0.35mm±0.48mm.
- The difference between group 1 and 2 was not statistically significant in any of the occasions as analysed with independent samples t test. However, better root coverage was achieved in group 1 when compared to group 2 at 24 weeks postoperatively as compared with values at baseline.

Table 1. Comparison between RD values at baseline and treatment endpoint

	GROUPS	Mean	Std. Deviation	t value	p value
Baseline	TEST GROUP	2.7500	.78640	0.395	0.695
	CONTROL GROUP	2.8500	.81273		
1 week	TEST GROUP	.0000	.00000	t value cannot be computed because the standard deviations of both groups are 0.	
	CONTROL GROUP	.0000	.00000		
6 weeks	TEST GROUP	.0500	.22361	1.798	0.080
	CONTROL GROUP	.2500	.44426		
12 weeks	TEST GROUP	.2500	.44426	0.677	0.503
	CONTROL GROUP	.3500	.48936		
24 weeks	TEST GROUP	.1500	.36635	1.463	0.152
	CONTROL GROUP	.3500	.48936		

Figure 1. Pre-Operative

Figure 2. Incision

Figure 3. Flap Reflection

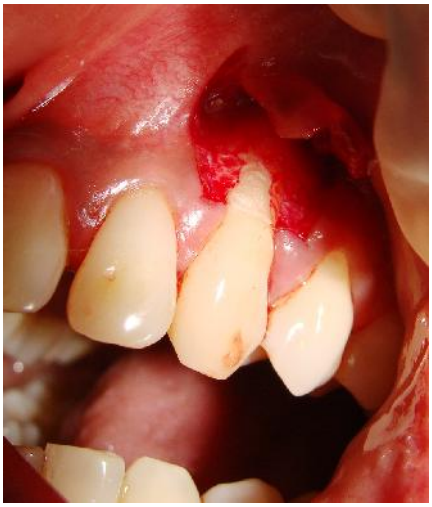

Figure 4. Placement of Amniotic Membrane

Figure 5. Suturing

Figure 6. 1 week Post-OP

Figure 7. 24 weeks Post-OP

DISCUSSION

The amniotic sac encloses the developing fetus through gestation and is composed of amnion and chorion tissue. Amnion lines the inner most portion of the amniotic sac and consists of a single layer of epithelium cells, thin reticular fibers (basement membrane), a thick compact layer, and a fibroblast layer (Figure 8). The basement membrane contains collagen types III, IV, and V and cell-adhesion bioactive factors including fibronectin and laminins. Data suggests the amnion basement membrane closely mimics the basement membrane of human oral mucosa. (Takashima *et al*., 2008) Of particular interest is the fact that this amnion layer possesses several types of laminins, with Laminin-5 being the most prevalent. Laminin-5 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. (Pakkala *et al*., 2002) Amniotic membrane is a composite membrane consisting of pluripotent cellular element which possesses the ability of transdifferentiation to other cellular elements of periodontium embedded in semipermeable membranous structure which makes it a suitable candidate for guided tissue regenration. (Lafzi and Farhani, 2007) It is an immunotolerant structure Amnion tissue contains growth factors that may aid in the formation of granulation tissue by stimulating fibroblast growth and neovascularization. (Koizumi *et al*., 2000) Additionally, the cells found within tissue exhibit characteristics associated with stem cells and may enhance clinical outcomes. (Toda *et al*., 2007) Amnion has shown an ability to form an early physiologic "seal" with the host tissue precluding bacterial contamination (Talmi *et al*., 1991) and multiple studies support amnion's ability to decrease the host immunologic response via mechanisms such as localized suppression of polymorphonuclear cell migration. (Hao *et al*., 2000) Amniotic membrane possesses certain biologic properties like reduced inflammation, diminished occurrence of adhesion and scarring, modulation of angiogenesis, promote wound healing, antimicrobial activity and promote epithelialisation. (Velez *et al*., 2010) Amniotic tissue has been used since the early 1900s for skin grafts, treatment of burns, and treatment of ulcerated skin conditions. (Gurinsky, 2009) More recently it has been used for temporary biologic dressings for full-thickness wounds (Robson *et al*., 1973), reconstruction of damaged or malformed organs, (Morton and Dewhurst, 1986) and prevention of tissue adhesion (Muralidharan *et al*., 1991). Additionally, use of amniotic tissue has been reported to decrease post-operative pain when used as a wound dressing. (Robson *et al*., 1973) Amnion tissue grafts have also been routinely used for the past decade in ophthalmologic surgery. Cryo-preserved amnion (Luanratanakorn *et al*., 2007) and dehydrated amnion (Memarzadeh *et al*., 2008) have demonstrated equivalent results to conjunctive autograft tissue in ocular reconstruction procedures. In the production of the amnion allograft used in this study, pre-screened, consenting mothers donate the amnion and associated tissues during elective cesarean section surgery. All donated tissue follows strict guidelines for procurement, processing, and distribution, as dictated by the United States Food and Drug Administration (FDA) and the American Association of Tissue Banks (AATB). These safety measures include testing for serological 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. 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. The aim of this clinical study was to document the use of processed dehydrated allograft amnion in the treatment of shallow-to-moderate mucogingival recession defects. Collected data and subjective observation indicates that the use of processed dehydrated allograft amnion provides good results in terms of root coverage, increased tissue thickness, and increased attached gingival tissue. Although statistically not significant results between both the groups, processed dehydrated allograft amnion demonstrated excellent esthetic results in terms of better root coverage, 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. The ability to self-adhere makes processed dehydrated allograft amnion an attractive option for multi-teeth procedures and recession defects in particularly hard to reach areas such as the molar region.

Conclusion

Based on the data collected in this study, processed dehydrated allograft amnion may provide an effective alternative to

autograft tissue in the treatment of shallow-to-moderate Miller Class I and II recession defects. Additionally, the self-adherent nature of the amnion allograft significantly reduced surgical time and made the procedure easier to perform relative to techniques involving the use of autograft or allograft dermis tissue. Although this study provides initially promising results for utilization of processed dehydrated allograft amnion in particular mucogingival defects, the limited number of patients, lack of controls, and short duration of this study warrants additional research be conducted to confirm the results of this study.

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