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Comparison of Amnion Allograft with Connective Tissue Graft for Root Coverage Procedures: A Double-Blind, Randomized, Controlled Clinical Trial

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Abstract

Objective: The aim of the present double-blind, randomized, controlled study was to evaluate and compare the efficacy of amnion allograft and connective tissue graft in covering denuded root surfaces.

Methods: Seventy-one teeth in 22 patients with gingival recession were treated randomly with coronally displaced flap plus connective tissue graft (control group, n = 29 recessions in 10 patients) or coronally displaced flap plus amnion allograft (test group, n = 42 recessions in 12 patients). The amount of root coverage and clinical parameters (probing depth, recession depth, clinical attachment level, recession width, gingival width, and papilla dimensions) were measured at baseline and at 3 and 6 months postoperatively.

Results: Average root coverage percentages after 6 months in the test and control groups were 67% (2.3 ± 0.289 mm) and 54% (2.24 ± 0.519 mm), respectively, with no statistically significant differences ($p = 0.054$). The changes in depth and width of recessions and in gingival width were significant 3 and 6 months after surgery compared to baseline ($p = 0.000$). Variations in the level of attachment and probing depths after 6 months were statistically significant in the test group compared to the control group ($p = 0.002$). Papilla dimensions were significantly correlated with root coverage ($p = 0.00$).

Conclusions: Amnion allograft might be a suitable alternative to connective tissue graft in procedures to cover denuded root surfaces and can reduce recession depth.

Key words: *transplantation, homologous, gingival recession, root coverage, gingival defects, gingival recession, periodontal surgery, soft tissue grafting*

Introduction

Gingival recession is relatively prevalent in the general population and may be associated with unfavorable esthetics, dentin hypersensitivity, and susceptibility to root caries. Of the factors contributing to gingival recession, trauma from toothbrushing is the most common (Lindhe *et al.*, 2008); other factors include anatomic variations (Joly *et al.*, 2007; McLeod *et al.*, 2009), smoking, oral habits, malpositioning and orthodontic tooth movement (Gray, 2000).

Exposed roots become susceptible to caries, because the contour of the gingival margin makes it difficult to control plaque (Oates *et al.*, 2003; Roccuzzo, 2002). Different surgical techniques have been introduced to treat gingival recession, including the free gingival graft (Lindhe *et al.*, 2008; Sullivan and Atkins, 1968), the coronally advanced flap (Allen and Miller, 1989), the coronally advanced flap with connective tissue graft (Langer and Langer, 1985), and various regenerative techniques, including the use of non-absorbable membranes (Prato *et al.*, 1992), absorbable membranes (Roccuzzo *et al.*, 1996), enamel matrix derivative (Rasperini *et al.*, 2000), and a platelet-containing gel with a coronally displaced flap (Keceli *et al.*, 2008).

Treatment with free gingival grafts is a painful procedure for patients because of denudation of the palate, and unpredictable results have been seen with respect to harmony with the adjacent tissues (Bouchard *et al.*, 2000). In contrast, the subepithelial connective tissue graft (SCTG) technique has an excellent prognosis, with good esthetic results. It is considered a standard approach compared to other root-coverage

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techniques (Harris, 2004); however, it has some disadvantages. It is time-consuming and traumatic for the patient. In addition, patients prone to gingival recession usually have thin palatal mucosa and are unable to provide connective tissue of adequate thickness (Muller and Eger, 2002). Limitations related to the removal of palatal mucosa (Paolantonio *et al.*, 2002) and the difficulty of obtaining connective tissue of uniform thickness, as well as the probability of retention of cells of palatal origin in the graft, which could result in improper color match, are additional disadvantages (Joly *et al.*, 2007; Paoloantonio *et al.*, 2002).

Recently, the use of allografts has been suggested for root surface coverage as an alternative to autogenous grafts. One of these products is acellular dermal matrix allograft (ADMA), which has been recommended as an alternative to autogenous grafts to cover denuded roots to prevent the need to harvest a CTG (Novaes *et al.*, 2001). However, subsequent studies of the amount of root coverage and esthetic results have yielded different findings, and insufficient data are available about long-term outcomes of the procedure (Moslemi *et al.*, 2011). Recently, a new allograft was introduced; it is derived from amniotic membrane (AM) and can decrease inflammation and scar formation to promote wound healing by encouraging cellular proliferation and differentiation. In addition, it has antimicrobial properties (Kothiwale *et al.*, 2009) and inhibits bacterial infection by inducing a rapid physiologic seal and promoting wound healing, as it also contains growth factors (Gurinsky, 2009).

Amniotic membrane is easily obtained and has been used in ophthalmologic surgeries, skin grafts, and treatment of burns and wounds (Solomon *et al.*, 2001; Tseng, 2001). It was initially used as a surgical material in skin transplantation (Davis, 1910) and for the treatment of burned or wounded skin (Sabella, 1913; Stern, 1913). Later, AM was used for the treatment of scleral lesions (De Rotth, 1940). Kim and Tseng (1984) revived its use in ophthalmologic surgery. Many studies have confirmed the antimicrobial and anti-inflammatory properties and efficacy of AM (Hao *et al.*, 2000; King *et al.*, 2007; Buhimschi *et al.*, 2004; King *et al.*, 2003; Kanyshkova *et al.*, 2001; Gomes *et al.*, 2005; Romero *et al.*, 1994).

The antimicrobial and anti-inflammatory properties of AM, along with the similarity between the basement membranes of AM and the oral mucosa (Meller *et al.*, 2000), might make it a suitable alternative to a CTG. Therefore, the present study compared success rates and clinical criteria for covering denuded roots using SCTG and subepithelial amnion allograft (SAAG, BioCover, Snoasis Medical, Denver, CO, USA) in Miller Class I and II gingival recessions. If a Class III gingival recession was present in teeth adjacent to the teeth under study, those teeth were also treated on the basis of patient demand and for ethical reasons.

Materials and methods

Study design and participants

The present double-blind, randomized, controlled clinical trial was conducted according to the guidelines of the Helsinki Declaration of 1975 (revised, 2000). The research protocol was approved by the Ethics Committee of the Dental Research Center of Tehran University of Medical Sciences. The study population consisted of patients referred to the Department of Periodontics, Tehran University of Medical Sciences, between March 2010 and August 2011, with at least one tooth with Miller Class I or II gingival recession. In addition, the following inclusion criteria were applied: 1) age over 18 years; 2) good oral hygiene; O'Leary oral plaque index < 15%; 3) no bleeding on probing based on the Ainamo and Bay index (1976); 4) presence of identifiable cementoenamel junction; 5) at least one Miller Class I or II gingival recession with a minimum depth of 2 mm on an incisor, canine, or premolar; if a class III recession was present adjacent to another tooth in the study, it was also treated and included on the basis of patient demand and for ethical reasons. Exclusion criteria were: 1) active caries lesions or restorations or crowns at the cementoenamel junction (CEJ); 2) smoking habit; 3) systemic conditions precluding periodontal surgery; 4) systemic conditions affecting the periodontium; 5) frenum pull at attached gingiva; 6) a history of mucogingival surgery in the area; 7) pathologic movement of the involved teeth.

The sample size was determined by a statistical power analysis. Considering a significance level of $\alpha = 0.05$ and $\beta = 0.2$ and assuming a standard deviation of 0.60 (based on a previous pilot study), a total sample size of at least 22 recessions in 20 patients (10 control and 10 test) would be required to detect a 1 mm intergroup difference in recession depth (RD).

Randomization of the patients and their assignment to intervention groups was carried out by an author who was blinded to the details of the study and surgical protocols (A.K.). The patients were numbered according to when they had presented to the department. After the patients' eligibility for enrollment in the study was confirmed, all the surgeries were done according to the patients' numbers. Concealed allocation was performed by sealed coded envelopes that were opened just before surgery to determine the test (SAAG) and control (SCTG) groups. To allow for possible dropouts, 24 patients were recruited. All surgeries were performed by one surgeon (A.A.R.G), who was blinded to the randomization sequences.

Periodontal parameters

Patients received oral hygiene instructions during a 2-week period before surgery, and scaling and root planing were carried out. All clinical parameters were measured before and after surgery by a calibrated postgraduate student who was blinded to the study

Figure 1. ***A. Measurements used to determine the success of the treatments provided in the study. PH indicates papilla height (black line), measured according to Haghighati et al.[38]; PW, papilla width (purple line); RW, recession width (red line); RD, recession depth (green line); and GW, gingival width (blue line). Panels B to G: (B) Preoperative image of a mandibular right canine with a Miller Class I recession; a coronally advanced flap (CAF) and a subepithelial amnion allograft (SAAG) were selected to treat the recession; (C) the site has been opened to fully view the defect; (D) SAAG placement after elevation of the split-thickness flap; (E) the pedicled flap has been coronally displaced and sutured to cover the graft; (F) root coverage at 3 months; (G) root coverage at 6 months. Panels H to L: (H) Preoperative image of a maxillary left canine and first premolar with Miller Class I recession and adjacent second premolar with Miller Class III recession, all of which were treated with a CAF and SAAG; (I) SAAG placement after elevation of the split-thickness flap; (J) the pedicled flap has been coronally displaced and sutured to cover the graft; (K) root coverage at 3 months; (L) root coverage at 6 months. Panels M to Q: (M) Preoperative image of a maxillary left canine presenting with a Miller Class I recession; this recession was treated with a CAF and a subepithelial connective tissue graft (SCTG); (N) the site has been opened, and the full extent of the defect can be seen; (O) connective tissue placement after elevation of the split-thickness flap; (P) root coverage at 3 months; (Q) root coverage at 6 months. Panels R to V: (R) Preoperative image of a maxillary left canine with a Miller Class I recession and adjacent premolars with Miller Class III recession; a CAF and SCTG were selected to treat the canine and the premolars at the request of the patient; (S) appearance after raising a flap; (T) connective tissue placement after the elevation of the split-thickness flap; (U) root coverage at 3 months; (V) root coverage at 6 months.***

protocol. All measurements were made using a standard UNC15 periodontal probe (Hu-Friedy, Chicago, IL, USA).

Baseline measurements (*Figure 1A*) included 1) O'Leary plaque index ; 2) bleeding on probing (Ainamo and Bay, 1976); 3) probing depth (PD) at the midbuccal aspect of the involved tooth; 4) RD: the distance between the CEJ and the gingival margin at the midbuccal aspect of the involved tooth; 5) recession width (RW): the distance between the mesial and distal papillae along the CEJ; clinical attachment level (CAL): the sum of PD and RD; 6) gingival width (GW): measured midbuccally from the gingival margin to the mucogingival junction using the roll test. The roll test is achieved by pushing the adjacent mucosa coronally with a dull instrument to mark the mucogingival junction (Bathla 2011); 7) gingival thickness: based on transparency of the gingiva [i.e., how easily the periodontal probe could be seen through the gingival margin while probing the buccal sulcus (De Rouck *et al.*,

Table 1. Baseline patient-related and defect-related characteristics

	SCTG (n = 29)	SAAG (n = 42)
Patient-related characteristics		
Age (mean ± SD, in years)	44.97 ± 6.23	47.79 ± 7.72
Sex		
Male	13 (45%)	17 (41%)
Female	16 (55%)	25 (59%)
Type of tooth		
Incisor	4 (14%)	14 (34%)
Canine	6 (20%)	12 (27%)
Premolar	19 (66%)	16 (39%)
Jaw		
Maxilla	8 (28%)	21 (50%)
Mandible	21 (72%)	21 (50%)
Defect class		
Class I	13 (45%)	21 (50%)
Class II	3 (10%)	3 (7%)
Class III	13 (45%)	18 (43%)
Defect-related measurements (mm)		
RD (mean ± SD)	4.21 ± 2.011	3.37 ± 1.478
RW (mean ± SD)	4.38 ± 0.852	3.89 ± 1.192
PD (mean ± SD)	1.74 ± 0.577	1.62 ± 0.642
CAL (mean ± SD)	5.98 ± 2.055	4.99 ± 1.403
GW (mean ± SD)	2.397 ± 1.2774	2.762 ± 1.6647

SCTG, subepithelial connective tissue graft; SAAG, subepithelial amnion allograft; RD, recession depth; RW, recession width; PD, probing depth; CAL, clinical attachment level; GW, gingival width.

2009)]; 8) papilla height (PH): measured according to Haghighati *et al.,* (2009); 9) papilla width (PW): the distance between the intersection of a line connecting the CEJ of the midbuccal aspect of two adjacent teeth and the mesial and distal aspects of the papilla *(Figure 1 A)*.

Examiner calibration

Eight non-study patients with gingival recession were recruited for calibration. The single designated examiner (S.S.) recorded PD, RD, and GW twice, with an interval of 24 hours between recordings. The intra-examiner repeatability for RD was determined, and the correlation coefficient was 0.94 ± 1 mm.

Study procedures

Clinical views of the procedure in several patients are shown in *Figures 1B to 1V*. The flap in both the test (SAAG) and the control (SCTG) groups was a full-thickness flap in the attached gingiva. This was followed by a split-thickness flap in the alveolar mucosa, with two releasing incisions on the sides of each target tooth that passed through the mucogingival junction. The adjacent papillae were de-epithelialized, and the denuded root surfaces were root planed. Tetracycline solution was placed on the denuded roots for 2 minutes and then rinsed off with saline solution. The SAAG and SCTG were placed on the root surface and the adjacent bone in the test and control groups, respectively *(Figures 1B to 1V)*. The connective tissue was stabilized with 4–0 vicryl sutures. After the graft had been placed on the root surface, the flap was displaced coronally to cover the graft and secured in place. A surgical dressing was used to cover the surgical sites. All the patients took 500 mg amoxicillin capsules for 7 days (three times daily), and two 400 mg ibuprofen tablets were given immediately after surgery before the local anesthesia wore off. In addition, 0.2% chlorhexidine mouthwash was administered for 6 weeks, and the subjects refrained from brushing the surgical site during this time. The

Table 2. Distribution of specimens treated with the SAAG and SCTG procedures in relation to patient gender and jaw location

	Treatment group			
	SCTG	**SAAG**	**All**	
Gender				
Female	16	25	41	$p = 0.808$
Male	13	17	30	$X^2_{(1)} = 0.133$
Total	29	42	71	
Jaw				
Maxilla	8	21	29	$p = 0.086$
Mandible	21	21	42	$X^2_{(1)} = 3.567$
Total	29	42	71	

Table 3. Comparison of clinical parameters between and within the SAAG (n = 42) and SCTG (n = 29) groups at baseline, 3 months, and 6 months postoperatively

Variable/ graft type	Time (mo)	Mean	SD	U	p^a	MR	df	X^2	p^b
RD									
	0	4.12	1.986	288	0.36	2.98			
SCTG	3	1.54	1.224	318.5	0.72	1.31	2	50.871	0.00*
	6	1.88	1.467	235.5	0.054	1.71			
	0	3.43	1.741			2.93			
SAAG	3	1.43	1.357			1.58	2	64.493	0.00*
	6	1.13	1.452			1.49			
RW									
	0	4.38	0.852	466	0.08	2.71			
SCTG	3	2.53	1.932	555	0.51	1.52	2	32.519	0.00*
	6	2.93	1.801	570	0.64	1.78			
	0	3.89	1.192			2.40			
SAAG	3	3.00	1.642			1.82	2	16.434	0.00*
	6	1.25	0.496			1.73			
CAL									
	0	5.98	2.055	448.5	0.058	2.98			
SCTG	3	3.31	1.795	533	0.36	1.29	2	47.145	0.00*
	6	3.82	1.593	344	0.00*	1.72			
	0	4.99	1.403			2.92			
SAAG	3	2.90	1.170			1.69	2	60.967	0.00*
	6	2.64	1.474			1.39			
GW									
	0	2.39	1.277	534	0.37	1.79			
SCTG	3	2.88	1.230	524	0.31	1.84	2	9.652	0.00*
	6	3.34	1.610	538.5	0.39	2.36			
	0	2.76	1.664			1.58			
SAAG	3	3.20	1.366			2.00	2	24.257	0.00*
	6	3.44	1.298			2.42			

df, degrees of freedom; X^2, chi-squared, MR, mean rank; SD, standard deviation; U, Mann-Whitney *U* test. [a]Between-group comparison; [b]within-group comparison; $*p < 0.05$

study assistants called the patients every day to remind them to use the analgesic tablets if there was pain and to inquire about any other side effects. The patients were recalled for reinstruction in hygiene and prophylaxis biweekly for 12 weeks and then monthly until 6 months post-surgery.

Table 4. Correlations between the amount of root surface coverage, papilla height and width, and gingival thickness

Factor	r	*p*
PH	–0.595	0.00*
PW	–0.529	0.00*
GT	0.079	0.26

PH, papilla height; PW, papilla width; GT, gingival thickness.

Table 5. Comparison of pain and edema in the SAAG (n = 12) and SCTG (n = 9) groups

	MR		U		Z		*p*	
Interval/group	**Pain**	**Edema**	**Pain**	**Edema**	**Pain**	**Edema**	**Pain**	**Edema**
2 hours								
SCTG	14.61	14.06	21.5	26.5	–2.312	–1.957	0.018	0.049*
SAAG	8.29	8.71						
6 hours								
SCTG	14.67	14	21.0	27.0	–2.357	–1.924	0.018	0.058
SAAG	8.25	8.75						
12 hours								
SCTG	14.61	11.28	21.5	51.50	–2.315	–0.178	0.018	0.862
SAAG	8.29	10.79						
1 day								
SCTG	13.72	11.22	29.5	52	–1.757	–0.143	0.082	0.917
SAAG	8.96	10.83						
3 days								
SCTG	14.11	10.89	26.0	53	2.038	–0.071	0.049	0.972
SAAG	8.67	11.08						
5 days								
SCTG	13.50	11.78	31.5	47	1.654	–0.499	0.111	0.651
SAAG	9.13	10.42						
7 days								
SCTG	14.17	10.83	25.5	52.5	2.151	–0.115	0.041	0.917
SAAG	8.63	11.13						

MR, mean rank; U, Mann-Whitney *U* test; $*p < 0.05$

All study variables were measured again at 3 and 6 months postoperatively. Root coverage percentage was calculated at 3 and 6 months with the following formula: (baseline RD – RD at 3 or 6 months)/baseline RD.

Evaluation of postoperative pain and edema

A visual analog scale was used to evaluate postoperative pain and discomfort. The scale consisted of a 10 cm line, with a range from 0 (no pain/edema) to 10 (severe and intolerable pain/edema). The patients were instructed in the use of the scale and were asked to mark the severity of their pain and edema on the scale at 2, 6, 12, and 24 hours postoperatively and again 3, 5, and 7 days after surgery. The number of analgesic tablets taken was also recorded.

Statistical analysis

According to the Kolmogorov-Smirnov test, the data were not distributed normally; therefore, the nonparametric Mann-Whitney *U* test was used to compare the two groups. The Friedman nonparametric test was used to compare data within each group. The Pearson correlation coefficient was used to assess correlations among the data.

Results

Table 1 presents the baseline patient- and defect-related characteristics. The test and control groups did not exhibit any significant differences in the proportions of men and women (p = 0.80) or in the number of teeth evaluated in maxillae versus mandibles (p = 0.86; *Table 2*).

Two patients from the control group were dropped from the study because they did not attend all follow-up appointments. Thus, 22 patients (10 in the control group and 12 in the test group) were available for all evaluations.

Twenty-nine recessions in the control group and 42 recessions in the test group were analyzed. The mean values for root coverage percentage after 3 months were 58% (test) and 62% (control). After 6 months, these values were 67% and 54% in the test and control groups, respectively, with no significant differences between groups at this time point (p = 0.054). Thirteen of 42 test group recessions (30.95%) and 7 of 29 control group recessions (24.13%) exhibited complete root coverage. Among the Miller Class III cases, 1 of 13 control sites (7.69%) and 5 of 18 test sites (27.77%) showed complete root coverage; thus, the test procedure was successful about four times more often than the control treatment for Class III recessions.

In both groups, RD decreased significantly compared to baseline after 3 and 6 months. The mean RD value in the test group decreased from 3.43 mm at baseline to 1.43 mm and 1.13 mm after 3 and 6 months, respectively, with a mean decrease of 2.3 mm (p = 0.000). In the control group, the mean RD value decreased from 4.12 mm at baseline to 1.54 mm and 1.88 mm after 3 and 6 months, respectively (p = .000; *Table 3*).

RW values had decreased significantly by 3 and 6 months postoperatively in both groups (p = 0.000). There was no significant difference between the groups (p = 0.64).

Compared to baseline, CAL improved significantly in both groups. In the test group, CAL decreased from 4.99 mm at baseline to 2.90 mm and 2.64 mm after 3 and 6 months, respectively; however, in the control group, it was 5.98 mm at baseline, decreased to 3.31 mm after 3 months, and then increased to 3.82 mm after 6 months (p = 0.000). After 6 months, significantly greater changes were seen in the test group versus the control group (p = 0.002). However, the 3-month changes were not significantly different between groups (p = 0.367).

The mean GW in the test and control groups increased significantly during the study period, with mean increases of 0.68 and 0.95 mm, respectively (p = 0.00). No significant difference was found between the groups (p = 0.399; *Table 3*).

A significant decrease and a non-significant increase were found for PD in the test and control groups, respectively. At 6 months, a significant difference was observed between the groups (p = 0.00), but the difference was not significant at 3 months (p = 0.471).

Regarding gingival thickness, 27 of 42 sites in the test group and 7 of 29 sites in the control group that had initially been labeled as thin were reclassified as thick after 6 months. No significant difference was seen between the two groups (*Tables 3 and 4*).

Correlations between root surface coverage, papilla height and width, and gingival thickness are shown in *Table 4*.

With respect to pain and edema, the values at 2 and 6 hours and 3 and 7 days postoperatively were significantly lower in the test group (p = 0.01). However, there was no significant difference between groups in the number of analgesics taken (p = 0.31; *Table 5*).

Discussion

This is the first clinical trial to compare the efficacy of SAAG for root coverage with that of SCTG, which is currently considered the gold standard (Chambrone *et al.*, 2009; McGuire and Scheyer, 2010) for the treatment of gingival recessions.

Amniotic membrane has been used extensively and shown to be effective for the treatment of burns, skin wounds, and ocular lesions (Solomon *et al.*, 2001; Meller *et al.*, 2000) and as a dressing to prevent inflammation in the ocular area (Nakamura *et al.*, 2004; Arya *et al.*, 2010; Meller *et al.*, 2011; Riau *et al.*, 2010; Said *et al.*, 2009). It facilitates epithelialization, preserves the normal epithelial phenotype, decreases inflammation, promotes angiogenesis, and decreases scar formation (Gomes *et al.*, 2005). However, the efficacy of this graft material for the coverage of denuded roots has not yet been evaluated.

The present study showed success with both SAAG and SCTG in covering denuded root surfaces. The mean RD during the study period decreased by 2.3 mm and 2.24 mm in the test and control groups, respectively. A significant decrease was observed in RD (0.3 mm) during the 3- to 6-month interval in the SAAG group. Any root coverage that is achieved after a month is considered a 'creeping attachment' (Borghetti and

Gardella, 1990; Goldman *et al.,* 1964). In a histological study in rabbits, fibroblasts and newly formed blood vessels were more numerous and epithelial thickness and collagen density were significantly higher in sites treated with AM (test group); however, polymorphonuclear neutrophil counts were lower than in the control group (Rinastiti *et al.,* 2006). The decrease in RD seen after 3 months in the present test group might be attributed to the improved capacity of SAAG to induce creeping attachment. Induction of fibroblast proliferation and the presence of vascular growth factors in the AM could accelerate angiogenesis and tissue maturation; these may be responsible for preventing necrosis of the coronal portion of the flap, resulting in better healing and more creeping attachment.

An increase in the thickness of the gingival tissues was observed in both groups. The collagen present in BioCover consists of types I, III, IV, V, and VII collagen, in addition to laminins and fibronectins (Riau *et al.,* 2010; Burman *et al.,* 2004). Thick gingival tissue can withstand trauma and any resulting recession, promoting creeping attachment and a more predictable surgical result (Hwang and Wang, 2006).

In the present study, there were no significant differences in the changes in RW between the two groups; significant improvements were observed in both groups, indicating root coverage success. This is in agreement with a recent study of Nickles *et al.,* (2010).

The CAL gain and decrease in PD after 6 months in the test group were significantly greater than in the control group. Probing depth in the SAAG group decreased by 0.37 mm after 6 months compared to baseline values; in contrast, in the SCTG group, this parameter increased by 0.19 mm. The greater CAL gains observed in the test group may indicate better efficacy of SAAG in improving these two important parameters clinically and statistically.

Amniotic membrane strongly resembles the oral mucosa basement membrane and contains different types of laminins, especially laminin-5, which plays an important role in the adhesion of gingival cells (Gurinsky, 2009; Arya *et al.,* 2010; Meller *et al.,* 2011; Riau *et al.,* 2010). Laminins can promote regeneration, accelerate tissue adhesion, and preserve tissues, all of which are key factors in improved healing of gingival lesions and might result in CAL and PD improvements. Furthermore, the antimicrobial agents that are present in AM, especially secretory leukocyte proteinase inhibitor I, lactoferrin, -defensin, and elafin (King *et al.,* 2003; Kanyshkova *et al.,* 2001; Gomes *et al.,* 2005; Romero *et al.,* 1994), might improve wound healing, especially in patients with poor oral hygiene.

Recent systematic reviews have shown no significant differences in RD, keratinized gingiva, and CAL changes between ADMA and SCTG (Cairo *et al.,* 2008; Chambrone *et al.,* 2010). In contrast, in the present study, CAL improved significantly in the SAAG group compared to the SCTG group, which might be attributed to the presence of growth factors in the SAAG compared to ADMA.

Despite a weak prognosis for root coverage in Miller Class III gingival recessions (Aroca *et al.,* 2010; Cueva *et al.,* 2004), there was a favorable decrease in RD in this type of recession: 1 of 13 (7.69%) control group sites and 5 of 18 (27.77%) test sites exhibited complete root coverage. The higher success rate of SAAG compared to SCTG in treatment of Miller Class III gingival recessions might be attributed to the anti-inflammatory and antimicrobial properties and presence of a large number of growth factors in the AM, especially epithelial growth factor, keratinocyte growth factor, β-fibroblast growth factor, and platelet-derived growth factor, all of which contribute to improvements in soft tissue adhesion to root surfaces (Riau *et al.,* 2010). Furthermore, this allograft contains some cytokines that affect progenitor cells, including nerve growth factor, brain-derived neurotropic factor, noggin, and activin, which may play vital roles in activating cells at the site to participate in regeneration and tissue maturation (Niknejad *et al.,* 2008). Studies evaluating the effect of growth factors on root coverage have shown that these agents can decrease postoperative hemorrhage and desquamation and promote tissue regeneration and reva scularization (McGuire *et al.,* 2009; Ishii *et al.,* 2012). The authors believe that a broader flap, which increases the number of blood vessels, could be the other factor in providing the positive outcomes seen here for Class III recessions.

The mean width of keratinized gingiva increased by 0.68 ± 0.366 mm and 0.95 ± 0.333 mm in the test and control groups, respectively. Many studies have emphasized the positive role of SCTG in increasing keratinized gingiva width (Joly *et al.,* 2007; Oates *et al.,* 2003; Cordioli *et al.,* 2001; Han *et al.,* 2008; Harris, 2002; Bouchard *et al.,* 1994), attributing this to its ability to induce epithelial cell differentiation at the recipient site (Karring *et al.,* 1975). The granulation tissue originating from the periodontal ligament, the tendency of the mucogingival junction to preserve its position after coronal displacement of the flap, and the amount of exposed graft after suturing might also play roles in increasing keratinized gingiva (Cordioli *et al.,* 2001; Han *et al.,* 2008). Also, the presence of keratinocyte growth factor, which might promote keratinization of epithelial cells and help the mucogingival junction maintain its position, may explain the efficacy of SAAG in inducing keratinization.

Patients in the SAAG group reported less pain and edema. Analysis of visual analog scale data showed that pain severity was significantly lower in the test group at the 2- and 6-hour and 3- and 7-day postoperative intervals. Analysis of edema ratings at 2 hours revealed significant differences between the two groups, with significantly less edema in the test group, clearly reflecting the improved antimicrobial and anti-inflammatory properties of SAAG compared to SCTG. In addition, the lack of a need for a second surgery in the oral cavity, along with the aforementioned important antimicrobial and anti-inflammatory factors in the AM, which also include elastase-inhibiting factor and interleukin-1 receptor antagonist (King *et al.,* 2003; Kanyshkova *et al.,* 2001; Gomes *et al.,* 2005; Romero *et al.,* 1994; Rinastiti *et al.,* 2006; Niknejad *et al.,* 2008; Oxlund *et al.,* 2005; Calvin and Oyen, 2007), might explain the reduced inflammation and pain in this group.

A positive significant correlation was noted between papilla dimensions (height and width of papillae) and the amount of root coverage in this study. This is in agreement with the findings of Haghighati *et al.* (2009) but conflicts with other studies (Berlucchi *et al.,* 2005; Saletta *et al.,* 2001); the differences might be attributed to the different techniques used for assessing papilla dimensions. Because the interdental papilla is considered the most coronal hematologic bed after de-epithelialization in mucogingival surgeries to which the root-covering flap is anchored, an increase in papilla dimensions might increase vascular exchange between the hematologic bed and the flap (Zucchelli *et al.,* 2006). Although papilla dimensions can influence the amount of root coverage, the surface area of the de-epithelialized portion might be more important than the height or the width of the papilla alone. Therefore, the SAAG might bea suitable alternative to the SCTG. However, further studies are necessary to measure clinical parameters with a constant reference point to facilitate a more accurate long-term evaluation of gingival changes. Additionally, a study that focuses on Miller Class I and II gingival recessions in a split-mouth design with a larger sample size might yield more accurate results.

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