Amniotic Allograft, A Possible Alternative to Free Gingival Graft in Improving Attached Gingiva Width: A Randomized Controlled Clinical Trial

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ABSTRACT

Background: Years of research have well demonstrated the pivotal role the attached gingiva plays in maintaining of periodontal health.

Objective: This study aimed to compare the efficacy of two technics, amniotic allograft and free gingival graft (FGG), in improving the attached gingiva width (AGW) around the teeth.

Methods: In this randomized controlled clinical trial study, 28 patients all in need of increased AGW were randomly halved and assigned to a test group receiving the amniotic allograft and a control group treated by a palatal FGG. Following the operation, the mean AGW, graft shrinkage, and color match were assessed and photographed at various intervals (1, 2, 6, and 12 weeks). The level of pain was also evaluated based on the visual analog scale (VAS).

Results: The AGW was not significantly different between the two groups in 2, 6 and, 12 weeks postoperatively (P=0.17, 0.73, 0.76 respectively). The same applied to the amount of shrinkage between the two groups at the intervals (p=0.38, p=0.57 and p=0.52 respectively). The amniotic allograft group was superior (not significantly) in terms of the color match (p=0.59, p=0.31 and p=0.18 respectively). However, it was found to have significantly lower VAS pain scores than did the control group ($p < 0.05$).

Conclusion: Application of the amniotic allograft could decrease the postoperative pain as well as discomfort and effectively increase the AGW. Therefore, given the drawbacks of FGG, the amniotic allograft can be considered as a viable alternative.

KEYWORDS: Attached gingiva; Amniotic membrane; Free gingival graft; Gingival augmentation

INTRODUCTION

There has been a long debate about whether the attached gingiva can make any significant contributions to the maintenance of periodontal health. Although some have associated the periodontal health merely with the optimal oral hygiene rather than the presence of attached gingiva, evidence suggests that if present, the attached keratinized gingiva can reduce the risk of gingival recession and mucogingival problems [1].

Accordingly, countless approaches have been adopted in order to increase the attached gingiva width (AGW), improving the periodontal health. Free gingival graft (FGG) is considered as a gold standard for gingival augmentation [2]. However, it suffers from some shortcomings such as the need for an additional donor site, the limited amount of the

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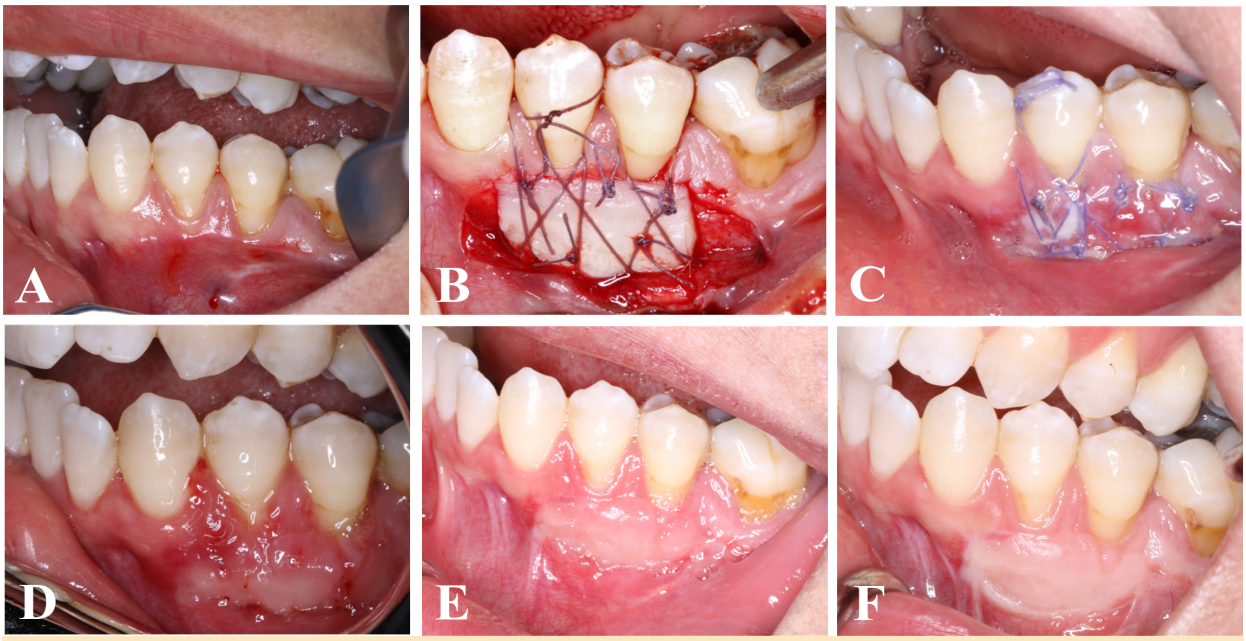

Figure 1: Clinical appearance of recipient site in control group: **A**) before surgery, **B**) immediately after surgery, **C**) one week after surgery, **D**) two weeks after surgery, **E**) 6 weeks after surgery, **F**) 12 weeks after surgery.

donor tissue, the time-consuming nature of graft harvesting, the postoperative pain, and the potential bleeding at the donor site. FGG may also provide undesirable esthetic results as the color match between the graft and the adjacent tissue at the recipient site is not predictable [3].

Thus, the above-mentioned complications have led clinicians to propose some alternatives such as acellular dermal matrix (ADM), extracellular matrix (ECM) membrane, bilayer collagen matrix (BCM), and living cellular construct (LCC). The alternatives partly address the concerns as they increase the AGW, result in an optimal color match and desirable esthetic appearance, and reduce the patients' morbidity [4], yet they still suffer from inflammatory responses (e.g. foreign body reactions), high costs, and the absence of long-term evidence regarding their stability [5]. Subsequently, there still exists an urge to propose other alternatives with more desirable outcomes.

Amniotic membrane (AM) is the innermost layer of placenta which consists of a thick basement membrane and avascular stromal matrix. Human AM has been used in different fields of medicine and dentistry [6]. The widespread use of this allogenic material in periodontal treatment is for its favorable properties: encouraging the optimal elasticity, producing bioactive peptides, growth factors and cytokines, enhancing migration of epithelial cells [7], increasing resistance to proteolytic factors [8], providing adequate permeability and optimal oxygenation of epithelial cells [9], inhibiting fibrosis and scar tissue formation [10], suppressing the host immune cells (and subsequent graft rejection) [11], possessing antibacterial and antiviral properties [12], preserving the pluripotent stem cells required for later differentiation[13], and being clinically user-friendly and cost-effective. The AM has been successfully used in various areas of periodontal therapy namely root coverage, treatment of intra-bony defects, treatment of furcation defects, ridge preservation, and biological dressing of oral ulcers [14].

Accordingly, this study aimed to assess the efficacy of the lyophilized AM as a versatile material to improve tissue management around the teeth.

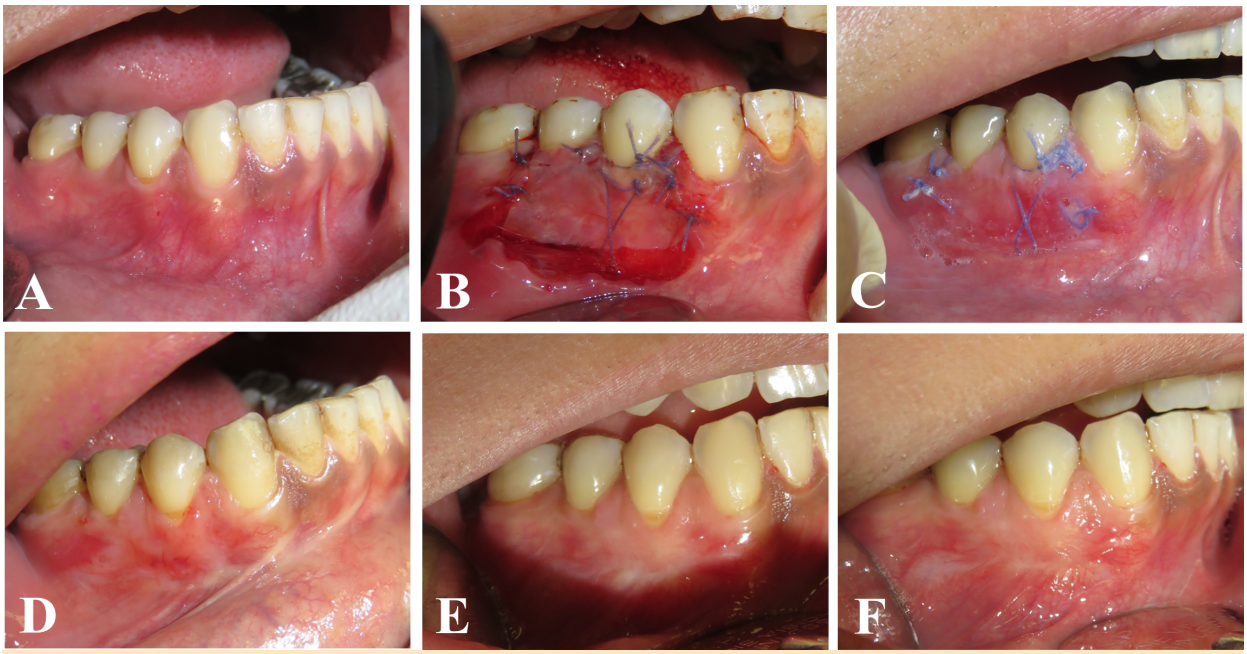

Figure 2: Clinical appearance of recipient site in test group: **A**) before surgery, **B**) immediately after surgery, **C**) one week after surgery, **D**) two weeks after surgery, **E**) 6 weeks after surgery, **F**) 12 weeks after surgery.

MATERIALS AND METHODS

This single blinded randomized control clinical trial study was based on the parameters registered at IRCT.ir (IRCT2016101621069N2) and was approved by the Ethics Committee of Tehran University of Medical Sciences (IR TUMS.VCR.REC1395.1608).

The participants were the patients referred to the Department of Periodontology, Tehran University of Medical Sciences, meeting the inclusion and exclusion criteria:

The inclusion criteria were as follows:

1) An age limit of minimum 18 and maximum 70

2) Presence of periodontal health

3) Presence of at least one region with attached gingiva ≤1 mm

4) Adequate plaque control with plaque index (PI) <20%

5) Patients' willingness to participate in the study

The exclusion criteria were as follows:

1) Presence of any systemic diseases or any medication known to affect/alter soft tissue healing

2) Smoking

3) Incompliance to postoperative instructions

The surgical procedure was thoroughly explained to all patients and those willing to participate signed informed consent forms. Randomization was based on a sealed envelope system; coded opaque sealed envelopes were opened right before each surgery in order that the patients could be randomly divided into two test and control groups.

Primary Clinical Parameters

Clinical parameters including the AGW, PI (according to O'Leary's index), gingival index (GI) and probing depth were recorded right before the surgical procedure. In order to record the color and texture of the surgical site at baseline, standard clinical photographs were taken from a 20-cm distance perpendicular to the tooth adjacent to the graft site.

Table 1: Gingival index, plaque index and pocket depth in the control and test groups.

	Time / Groupe	Baseline	First week	Second weeks	6th weeks	12th weeks
Gingival Index	Control (FGG) Mean ± SD	1.14±0.36	1.86±0.36	1.36±0.50	0.86±0.36	0.71±0.61
	Test (AM) Mean ± SD	1.07±0.47	1.93±0.27	1.29±0.47	0.64±0.50	0.64±0.063
	Inter group analysis (P-value)	0.804	0.55	0.69	0.20	0.74
Plaque Index	Control (FGG) Mean ± SD	19.36±1.15	37.29±5.38	33.64±4.25	23.86±2.14	24.07±3.59
	Test (AM) Mean ± SD	18.64±1.60	31.07±4.39	27.86±3.72	22.21±4.23	22.21±3.24
	Inter group analysis (P-value)	0.019	0.003	0.001	0.021	0.16
Probing Pocket Depth	Control (FGG) Mean ± SD	1.86±0.53	-	-	-	1.93±0.47
	Test (AM) Mean ± SD	2.14±0.77	-	-	-	1.93±0.73
	Inter group analysis (P-value)	0.27	-	-	-	P>0.999

Preparation of Human AM

The AM was procured from the placenta of healthy pregnant women undergoing elective Cesarean section. Those with immunodeficiency, transmissible diseases or infectious diseases were excluded. The graft was prepared and preserved as described by Kim[15]. Then, the membrane was freeze-dried at -80 °C, packed in a two-layer polyethylene bag, and transferred to the Iranian Atomic Energy Agency in a radiation box for sterilization.

Surgical Procedure

After administration of local anesthesia (Lidocaine HCL 2% with Epinephrine 1:100,000), an incision with blade No.15c was made along the mucogingival line with the required length and a partial thickness mucosal flap was elevated. Two vertical releasing incisions (10 mm in length) were made at two ends of the primary incision line. Next, the mobile tissues and tissue tags were removed by a pair of scissors to create a stable, non-mobile periosteal bed with no muscle attachment. Then, the flap was sutured (absorbable 4-0 vicryl sutures) at the new vestibular depth.

In the control group (FGG), the graft size was determined by a tinfoil placed over the recipient site according to Sullivan and Atkins[16]. After performing the local anesthesia (Lidocaine HCL 2% with Epinephrine 1:100,000), a graft (1.5 mm thick) was harvested from the palate (premolar area) and the area was sutured with 4-0 silk sutures (SUPASIL, Braided silk, Iran) and covered with a periodontal dressing. The harvested graft was stabilized at the recipient site using 4-0 vicryl sutures (SUPABON, Polyglycolate coated, Iran). A standard photograph was taken from the recipient site.

In the test group (the amniotic graft), a template was applied to determine the size of the graft. Next, the amniotic allograft was stabilized at the recipient site with absorbable 4-0 vicryl sutures. A standard photograph was obtained from the recipient site.

The postoperative instructions included: 1) rinsing 0.2% chlorhexidine gluconate mouthwash (Behsa, Iran) twice a day for two weeks 2) taking an analgesic (500 mg acetaminophen) to control pain (2 tablets taken immediately after surgery and 4 tablets taken on a daily basis within the first 48 hours following operation) 3) taking a systemic anti

Table 2: Attached gingiva width (mm) in control and test groups at 2, 6 and 12 weeks after surgery.

	Attached Gingiva Width (mm)		
Time / Groupe	Second weeks	6th weeks	12th weeks
Control (FGG) Mean ±± SD	5.18±1.84	4.57±1.79	4.25±1.55
Test (AM) Mean ±± SD	6.07±1.50	4.79±1.44	4.43±1.47
Inter group analysis (P-value)	0.17	0.73	0.76

biotic (500 mg amoxicillin, tid) for one week, 4) avoiding chewing hard food, 5) refraining from brushing teeth and mechanical trauma at the surgery site.

Postsurgical appointments were scheduled for 1, 2, 6, and 12weeks after the surgery and photographs were taken from the recipient sites on the same intervals. The sutures were removed after 2 weeks (Fig 1 and Fig 2).

Postsurgical Assessment of Clinical Parameters

AGW: The mean AGW was recorded (in millimeters) adjacent to each tooth at the recipient site 2, 6, and 12 weeks after the surgery.

Graft shrinkage: The surface area of the graft was measured in square-millimeters using a UNC-15 probe in 2, 6, and 12 weeks. Where the shape of the graft site was asymmetrical and complex, it was hypothetically divided into simple geometric shapes to avoid any complexity in measurement of the surface area. In order to calculate the shrinkage level, the measured values during the weeks 2, 6, and 12 were compared with the primary surface area of the harvested FGG or amniotic allograft.

Color match: The color match of the recipient site with the adjacent healthy tissue was scored at 1, 2, 6, and 12 week intervals: Score 1: Less than 50%; score 2: 50%; score 3: More than 50%; score 4: Perfect match.

Pain: Levels of pain were evaluated, using a 0-10 visual analog scale (VAS) where 0 and 10 indicated no pain and maximum imaginable pain respectively. The levels of pain and the number of analgesics taken per day were recorded for the patients both on the day of the surgery and during the first and second weeks. Meanwhile, the patient's PI and GI were evaluated both at baseline and at 1, 2, 6, and 12 week intervals. The pocket probing depth (PPD) was measured 12 weeks after the surgery.

Calibration

The photographs were observed by three periodontists at 1, 2, 6, and 12 week intervals. The correlation coefficient values were calculated and the inter-examiner reliability was found to be acceptable.

Sample Size Calculation

According to Sanz *et al.* [17], the sample size was set at n=28 with 14 samples in each group (The statistical values were α=0.05, β=0.2, μ1=2.6, μ2=1.6, and the average SD=0.9)

Statistical Analysis

The data were analyzed using SPSS version 21 (SPSS Inc., Chicago, Illinois). Continuous quantitative variables were compared by a t-test, while the Mann-Whitney test was used to compare the ordinal variables. The level of statistical significance was set at P=0.05.

RESULTS

The study was conducted on a total of 28 patients (4 males and 24 females) aged between 18 to 59 years (42 in average).

GI was not significantly different between the two groups 1 (P=0.55), 2 (P=0.69), 6 (P=0.20) and 12 (P=0.74) weeks (Table 1) after the surgery.

PI in the amniotic allograft group was significantly lower than it was in the FGG group at

Table 3: Graft shrinkage (%) and color match scores in control and test groups at 1,2, 6 and 12 weeks after surgery.

	Groupe \ Time	First week	Second weeks	6th weeks	12th weeks
Graft Shrinkage (%)	Control (FGG) Mean ± SD	-	41.95±17.49	50.27±17.87	55.08±15.91
	Test (AM) Mean ± SD	-	29.49±12.22	46.77±14.11	51.15±15.62
	Inter group analysis (P-value)	-	0.38	0.57	0.52
Color Match	Control (FGG) Mean ± SD	0.79±0.80	1.43±0.76	2.00±0.88	2.07±0.83
	Test (AM) Mean ± SD	0.93±0.73	1.79±0.70	2.43±0.51	2.50±0.52
	Inter group analysis (P-value)	0.59	0.31	0.18	0.16

the first (P=0.003) and the second (P=0.001) weeks. During the week 12, both groups experienced a rise in PI values recorded at the baseline (Table 1).

No significant changes were observed in terms of PPD between the two groups (P=0.27) (Table 1).

Each surgery in the amniotic allograft group took significantly a shorter period of time than it did in the FGG group (39.50±6.91 minutes compared to 63.79±12.27 minutes, P<0.001).

The AGW was not significantly different between the two groups in 2 (P=0.17), 6 (P=0.73) and 12 (P=0.76) weeks (Table 2).

There was no significant difference in the amount of shrinkage between the two groups in 2 (P=0.38), 6 (P=0.57) and 12 (P=0.52) weeks (Table 3).

Among the parameters evaluated on photographs, the color match was superior (yet not significant) in the amniotic allograft group 1 (P=0.59), 2 (P=0.31), 6 (P=0.18) and 12 (P=0.16) weeks after the surgery. There were gradual improvements in color match in both groups at the week 12 (Table 3).

Table 4 shows the VAS pain scores of patients on the day of surgery and the mean values in the first and second weeks in the two groups. All VAS pain scores in the test group were significantly lower than the corresponding values in the control group (P<0.05).

DISCUSSION

This single blinded randomized control clinical trial compared the impact of dehydrated amniotic allograft with FGG on increasing the AGW. To test the same hypothesis, a large number of studies have assessed methods of soft tissue regeneration around teeth; however, no consensus has been reached on an ideal alternative to autogenous grafts for this purpose. Despite the significant improvements in the AGW 6 weeks after the surgery, both groups saw a decrease during the week 12, when the mean AGW was 4.43±1.47 mm in the amniotic allograft and 4.25±1.55 mm in the FGG group; the difference in this respect was not significant between the two groups.

Since amniotic membrane contains types I, III, IV, V and VII collagens, laminin, and fibronectin, the success of this graft could be associated with the presence of a collagenous structure, mimicking the gingiva, and the basal lamina in particular. It also induces the

Table 4: AVisual analog scale pain scores in control and test groups at first day, 1 and 2 weeks after surgery.

Groupe \ Time	Visual Analog Scale Pain Scores		
	Second weeks	6th weeks	12th weeks
Control (FGG) Mean ± SD	6.57±1.09	4.43±1.28	1.86±0.77
Test (AM) Mean ± SD	5.29±0.61	3.29±0.91	1.29±0.61
Inter group analysis (P-value)	0.001	0.01	0.03

proliferation of fibroblasts and contains the vascular endothelial growth factor, enhancing angiogenesis and tissue maturity [18]. Moreover, the presence of different growth factors, especially epidermal, keratinocyte, fibroblast, and platelet-derived ones can significantly enhance soft tissue adhesion [18].

Nevins et al. [19] designed a split-mouth randomized clinical trial on 6 patients with inadequate AGW bilaterally to compare the efficacy of DynaMatrix extracellular membrane with FGG. They reported an average increase in the keratinized gingiva width in ECM (of 2.60±1.10 mm) and in FGG group (5.30±1.30 mm). Thoma et al. [20] compared the AGW and graft shrinkage between FGG and allograft groups and concluded that allografts resulted in an acceptable gain of the AGW. However, FGG was superior to allografts with a borderline significant difference. They also reported that allografts (acellular dermal matrix/human fibroblast-derived dermal substitute) experienced a greater level of shrinkage than FGG in 6 and 12 months. A systematic review by Baryl et al. [21] reported that FGG caused a greater increase in the AGW than do acellular dermal matrix and other alternatives to soft tissue grafts.

The current results showed that when placed more apically (than the mucogingival junction) to increase the AGW, dehydrated amniotic allograft had an advantage over other allogenic materials since with no considerable difference in the levels of shrinkage in FGG, it acceptably increased the AGW. Moreover, it should be noted that unlike FGGs, the amniotic membrane has no limitation regarding the size and can be simultaneously used in different regions to increase the AGW. Needless to say, a long-term stability assessment is still required for further studies.

This study also evaluated the duration of the surgery (39.50 minutes in the test and 64.00 minutes in the control group in average). Schmitt et al. [22] compared Mucograft with FGG and reported that the duration of the surgery in Mucograft group (65.11±15.36 minutes) was significantly and expectedly shorter than that of the FGG group (84.33±14.23 minutes) because the amniotic graft had been prepared in advance. This could be also explained by the uncomplicated and quick shaping of amniotic allografts, their adaptation with the recipient site, and their less mobility during the suturing phase. The subsequent reduction in the surgical time results in higher patients' cooperation and increased accuracy of the surgical procedure.

The color match at all time-points was higher (not significantly though) in the amniotic allograft group than it was in the FGG group. In the final follow-up session, half of the patients in the test group showed a perfect color match and the other half showed the >50% color match, implying that amniotic membrane provides acceptable esthetic results. As the amniotic membrane possesses a gingiva-like collagenous structure, it can decrease the production of TGF-β and expression of receptors for fibroblasts, minimize the scar tissue formation, and encourage optimal color match [18, 23].

Previous studies have mainly reported superior color match when allografts were used instead of autogenous grafts. In line with our findings, Scarno et al. [4] used the acellular dermal matrix to increase the AGW and reported an acceptable color match in 3 months. Nevins et al. [19] reported that 13 weeks after

the surgery, the ECM membrane had significantly higher levels of color match and tissue blending than did the autogenous gingival graft. Schmitt et al. [22] showed that in the Mucograft group, the grafted soft tissue had an acceptable clinical appearance comparable to that of the adjacent gingiva, while the grafted area in the FGG group was still recognizable after 5 years.

The heterogeneity regarding the significant superiority of allografts to FGGs in terms of color match may be due to the heterogeneity in methods of assessment and the use of rather subjective qualitative measures. Moreover, to assess the appearance of the graft area, both contour of the graft and color match should undergo evaluations. FGGs often have irregular contours and inappropriate blending with the adjacent soft tissue even in the presence of optimal color match. They often remain more prominent than the adjacent tissue, whereas the areas grafted with amniotic allografts are not prominent. Future studies should focus on the contour and tissue blending of amniotic allografts and FGGs in the long-term.

Although slightly superior in the test group, the GI was not significantly different between the two groups at the assessed time points. The processes of healing and resolution of inflammation were acceptable in both groups. Rinastiti et al. [24] histologically evaluated the application of amniotic membrane grafts to gingival ulcers in rabbits, where the samples were obtained from the site 1, 3, 5, 7 and 10 days after the transplantation. The results showed that the number of fibroblasts and the rate of angiogenesis were higher and the number of polymorphonuclears was lower in the transplanted group compared to the control group. Also, the thickness of epithelium and density of collagen were reported to be significantly higher in the transplanted group. These findings indicated that the use of amniotic membrane enhanced the healing process and reduced the inflammation after the surgery. The unique properties of amnion (low immunogenicity, anti-inflammatory and anti-microbial activity as well as the mechanical and physiological properties comparable to those of gingiva) account for the optimal soft tissue healing and decreased inflammation after the surgery [25]. The amniotic membrane is abundant with a number of anti-inflammatory and antimicrobial factors such as elastase inhibitors, IL-1α, IL-1β, matrix metalloproteinases, secretory leukocyte protease inhibitor, IL-1 receptor antagonist, B-defensin, elafin, and lactoferrin [22, 26, 27]. The B-defensins present in amnion makes a large group of antimicrobial peptides released by epithelial cells and leukocytes on mucous membranes, which play important parts in innate immunity [17]. The secretory leukocyte protease inhibitor and elafin have anti-inflammatory and antimicrobial properties; they are parts of innate immunity and decrease the susceptibility to infection [28, 29]. Lactoferrin is a multi-functional protein with anti-oxidative and iron-chelating properties. It inhibits IL-6 and effectively eliminates inflammation, infection, and pathogenic microorganisms [30]. The presence of antibacterial agents, especially in patients with poor oral hygiene, can prevent unusual healing patterns and decrease the rate of inflammation.

Amnion contains cytokines such as nerve growth factor (NGF), prednisone factor (PDNF), noggin, and activin that influence the progenitor cells and induce the cells present at the site to participate in the process of healing and consequent tissue maturation [31]. Concerning the immunogenicity of amnion, it has been suggested that the decrease in immune reactions and immune intolerance may stem from the absence or insignificant expression of human leukocyte antigen (HLA) class A, B and C antigens and insufficient expression of major histocompatibility complex (MHC) class II antigens including HLA-DP, HLA-DQ, and HLA-DR. Thus, these allografts do not cause cytotoxic reactions and prevent the proliferation of lymphocytes [32].

This study found that the test group experienced lower levels of pain 1 day, 1 week and 2 weeks after the surgery with the maximum difference in the levels of surgical pain on the day 1. The pain score reported by patients in amniotic allograft group did not exceed 6 (out

of 10) during the study. This finding is clearly attributed to the absence of donor site morbidity as well as the anti-inflammatory and antimicrobial effects of amniotic allograft.

It has been recently found that the use of allografts could mitigate postoperative pain. Sanz et al. [17] compared Mucograft and autogenous graft around dental implants and reported that the pain score was 2.30 to 2.39 in the test and 4.01 to 8.50 in the control group during the first 10 days. The finding was also reported by Nevins et al. [21] and McGuire et al. [33]. As the use of amniotic allograft lowers the levels of pain and shortens the duration of the surgery, it may bring about higher patient satisfaction, leading to patients' cooperation for future periodontal treatments.

Within the limitations of this study, it could be concluded that amniotic allografts effectively improve the AGW. However, studies with longer follow-ups are required to assess the stability of the results. Moreover, the use of amniotic allografts can significantly decrease the level of postoperative pain and discomfort of patients.

CONFLICTS OF INTEREST: None declared.

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